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STRUCTURE AND DEVELOPMENT OF THE SPOROPHYLLS AND SPORANGIA OF ISOETES

A DISSERTATION SUBMITTED TO THE FACULTIES OF THE GRADUATE SCHOOLS OF ARTS, LITERATURE, AND SCIENCE, IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

BY
R. WILSON SMITH

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APRIL, 1900

THE STRUCTURE AND DEVELOPMENT OF THE SPOROPHYLLS AND SPORANGIA OF ISOETES.

CONTRIBUTION FROM THE HULL BOTANICAL LABORATORY. XVIII.

R. WILSON SMITH.

(WITH PLATES XIII–XX)

FEW plants have excited more interest than Isoetes, a small genus of about fifty species, which has been variously classified, and the histology and development of which have been described in the most contradictory manner. It was with the purpose of obtaining, if possible, some data by which to clear up its homologies and relationships, and especially of examining the foundation of a claim made in recent years of its being the point of contact between monocotyledons and vascular cryptogams that the following investigation was undertaken.

The intention at first was to have the work include not only the reproductive parts of the sporophyte, but also the development of the female gametophyte and of the embryo. But so small a proportion of the spores was found capable of germination that the study of the prothallium had to be abandoned; and my

observations of the embryo agree so closely with those of Professor Campbell (4) that it did not seem worth while to publish any drawings. One difference may be noticed here and this part of the subject may be dismissed at once. Campbell says that but three archegonia are formed at first, and only in case none of these are fertilized do others appear. I have found new archegonia appearing even after three embryos had begun to develop, two of which had made considerable growth. It thus appears that sometimes new archegonia may arise even after fertilization.

The species selected for study were *I. echinospora* and *I. Engelmanni*, the former of which was examined more carefully. The material was collected by Mr. Raynal Dodge, of Newburyport, Mass. Part of it was fixed at once, and part after it had been cultivated for some time in the laboratory. The fixing reagents employed were 1 per cent. chrom-acetic acid, and Flemming's weaker solution. After remaining twenty-four hours in one of these fluids it was washed twenty-four hours in water and transferred through graded alcohols and chloroform or xylol to paraffin. The sections were cut 5, 10, or 15μ in thickness and stained in the ordinary way on the slides. Delafield's hæmatoxylin and erythrosin, safranin and gentian violet, Heidenhain's iron-alum-hæmatoxylin, and cyanin and erythrosin were all used with good results except in the case of the megaspore mother cell.

THE STEM.

The technique which is best adapted to an investigation of the development of the sporangia is not very suitable for an examination of the histology of the stem. Accordingly I have not attempted to make an exhaustive study of the latter, or of the vascular bundles of the leaf. Still the arrangement of the stem tissues is so peculiar that a few remarks will not be out of place. There is probably little variation in this respect in the different species. *I. echinospora* and *I. Engelmanni* agree very closely with *I. lacustris* as figured and described by Farmer (1), whose account is the latest and best dealing with the structure

of the vegetative organs. The center of the stem is occupied by a mass of short spiral and reticulated tracheids interspersed especially near the periphery with less numerous parenchymatous cells. The peripheral parenchyma is not sufficiently aggregated or continuous to form a xylem sheath such as occurs in the leaves. The xylem region is surrounded by a ring of tabular cells of glistening white appearance, thick-walled and empty towards the center but thin-walled towards the outside and more or less banded with incomplete layers of starch-containing cells. The cells are arranged in pretty regular radial rows, whether examined in longitudinal or transverse section. This ring is usually designated the prismatic layer, and very frequently, after Russow (1), the phloem. Russow claimed to have traced a continuity between the prismatic layer and the phloem of the leaf. I have not been able to satisfy myself of any organic continuity, but even did it exist it seems to me very questionable whether that would be a sufficient reason to justify Russow's view. No clearly defined sieve-tubes, the essential elements of the phloem, have ever been found either in the stem or in the leaf; and besides the inner cells of the prismatic zone are known to become secondarily thickened and transformed into xylem tracheids. The cells marked *o* in *fig.* 5 are in this process of transformation. Does not this indicate, if not a xylem character, at least the undifferentiated nature of the prismatic cells? A transformation of phloem into xylem would be, to say the least, an anomaly. In view of these difficulties to which may be added another — the relation to the cambium — it seems better to drop this application of the word phloem until its justification shall be established on physiological grounds. A small portion of the prismatic layer is shown in *fig.* 5.

Immediately outside the prismatic layer and indistinguishable from it except in the staining and size of the cells, is a zone of meristem which by its active division gives origin outwardly to an immense mass of cortex, and internally adds slowly to the prismatic layer. This zone is the so-called cambium. Its cells contain deeply staining plasmic contents in addition to starch

(*fig. 5*). Whether or not the dividing cylinder is more than one cell-layer in thickness I could not determine.

The cortex is very bulky and consists throughout of isodiametric parenchymatous cells abundantly filled with starch together with a little oil. The cells undergo no divisions, but as they are forced outward by the activity of the cambium they increase very greatly in size (*figs. 6* and *7*). In this way by the growth of the cells in all directions the cortex expands upwards and downwards as well as outwards, and as a result carries up the older leaves to a height considerably above the stem apex. This is illustrated in *fig. 68*, in which the unbroken lines represent the form which the cortex would take if its cells underwent no enlargement as they are pushed out from the cambium, and the dotted lines the form and dimensions which it actually assumes.

The stem apex lies at the base of the conical depression formed in the manner just explained. In small plants it is distinguishable in longitudinal sections as a slight elevation (*fig. 3*); in older plants it is merely a flattened area between the bases of the young leaves (*fig. 4*).

The method of growth of the apical meristem was first correctly described by Hegelmaier (2). Hofmeister (1) had erroneously ascribed it to the segmentation of an apical cell, having been led to that conclusion probably by too exclusive study of young plants. There is neither an apical cell nor such a group of initials as might result from the division of a rectangular apical cell like that of the Marattiaceæ. Only two or three layers of cells show their meristematic nature by their contents. The superficial layer appears to divide only in an anticlinal direction except when young leaves are about to be formed; but this layer, as Hegelmaier showed, can on no account be regarded as a dermatogen.

Although all the species of Isoetes are perennial, only a small portion of the plant persists from year to year. The roots, the leaves, and the bulky cortex are shed or decay annually, and are as often renewed from the stem apex and the meristematic zone which surrounds the small central permanent cylinder.

THE LEAF AND LIGULE.

In its earliest recognizable form the leaf rudiment seen from above is a crescent-shaped band of meristematic cells, curved about the stem apex. Sections show that it arises from the superficial cells of the stem apex, and is soon pushed up into a low broad mass, highest in the middle and inclined inwards. The ligule appears very early, and the leaf becomes distinguishable into a proximal part somewhat triangular in section and destined to bear the sporangium, and a distal part approximately circular in section and destined to become the chlorophyllous region. In correlation with the rapid development of the sporangium, the growth of the leaf is at first almost confined to the basal region. Compare, *e. g.*, the three leaves shown in *fig. 8*; tranverse sections would show the rapid growth of the basal region in a still greater degree. This region continues to widen as the leaf is pushed outward, by the formation of new leaves and the diametral enlargement of the stem; but longitudinally, except for a slight addition below the sporangium, there is only sufficient growth to accommodate the sporangium, velum, and ligule.

When the sporangium is well under way the region of rapid multiplication and growth of cells is transferred to the part above the ligule. The cells here are arranged with beautiful regularity, and growth is so rapid that this soon becomes the most prominent part of the leaf. The maximum diameter, so far as the number of cells is concerned, is speedily attained, and growth thereafter is only in the longitudinal direction. At first every part of the leaf rudiment is meristematic, but in a short time the apex passes over into permanent tissue. This change into permanent tissue progresses gradually downward until finally the whole leaf is involved. For some time a region of ever narrowing extent above the ligule continues in active division, but there is present no sharply marked or persistent meristematic zone, as seems to be implied in Farmer's account. The leaf is still quite small when all cell divisions have practically ceased, and its further elongation, which may amount to

400 or 500 per cent., is accomplished by the growth of the individual cells.

The formation of the air cavities is interesting, since it is comparable in some respects to the differentiation of trabecular and sporogenous tissue in the sporangium. In a leaf such as is represented in cross section in *fig. 9*, there is yet no sign of the air chambers. Increase of diameter is actively going on and the whole leaf is still meristematic. In the leaf shown in *fig. 10* the position of the future air chambers is indicated by four symmetrically placed groups of cells which have lost most of their contents. The peripheral cells of the leaf, the central cells, and four radiating bands which appear in cross section as spokes arranged in the form of the sign + continue to grow and are distinguishable by their larger more densely filled cells. Stained with Delafield's haematoxylin and erythrosin these cells show deep red cytoplasmic contents and large nuclei in which the red staining predominates; while in the areas which are to become air chambers the cytoplasmic contents have almost entirely disappeared, but the nuclei still retaining their chromatin stain intensely with haematoxylin. When only a nuclear stain is employed, such as iron-alum-haematoxylin, the four non-protoplasmic areas are rendered very prominent by their black nuclei. Longitudinal sections show that the regions which are thus sharply distinct in cross section run lengthwise of the leaf in unbroken bands from just above the ligule nearly to the apex, and there are as yet no air cavities.

The air chambers are formed lysigenously. The growing tissues generate a tension in the empty cells, and as a result these are ruptured irregularly, and small cavities appear, separated by diaphragms or plates of cells extending across from the central to the peripheral growing regions. As the leaf elongates, the air cavities increase in size, while the diaphragms drawn farther and farther apart lose their protoplasm to the surrounding cells. When once this splitting into diaphragms and cavities has occurred, it is not repeated; there remains no meristem in which they may be generated. Occasionally single diaphragms

of unusual thickness may be again ruptured, but no considerable increase in their number ever occurs after their first formation.

It is easy with the low power of the microscope to count the diaphragms in leaves floating upon a little water on a slide. The usual number is from fifty to seventy, and is quite as many in young leaves three fourths of an inch long as in leaves fully formed. It is instructive, too, as proving the absence of a definite meristematic zone, to count the average number of superficial cells which intervene between the diaphragms. In very young leaves this is from three to six or eight throughout the whole length, but in older leaves it is much greater, varying from twelve to twenty in the tip region to forty to sixty in the middle and basal regions, which remain longest in the meristematic condition.

The diaphragms, I think, are quite functionless, and their existence merely incidental to the manner of origin of the air chambers. They are too delicate to serve for mechanical support, which is sufficiently secured by the four longitudinal bands already described. The position of the air chambers and longitudinal bands between them in relation to the axis of the plant is always the same as that indicated in *figs. 10, 11, 44*. Near the ligule the air spaces are less regular, and instead of four of them symmetrically placed we find many irregular ones. Behind the sporangium the dorsal longitudinal band of living cells, and sometimes the two lateral ones, are well marked, but there are no large distinct air spaces. The vascular bundle of the leaf is as characteristic as that of the stem. My observations, referring chiefly to the changes of form of the bundle, were made with the view of discovering whether there is any definite relation between it and the sporangium or the ligule, and whether it presents any evidence that the leaf of Isoetes has been reduced from a more complex type. The leaf trace can first be recognized in the base of the young leaf and in the stem region below it towards the central bundle. The xylem elements are first differentiated, and consist of five or six tracheids grouped into a cylinder and surrounded by a sheath of parenchymatous cells with dense

contents. These parts can be traced later to the corresponding parts of the axial bundle. Behind the sporangium the xylem spreads out into a broad band in which the amount of xylem parenchyma is greatly increased, and the tracheids are in five or six scattered groups. Above the sporangium the xylem contracts again into a cylinder, and lies between the cornua of the ligule base. A more striking change occurs above the ligule where the xylem elements suffer an extreme diminution, there being in that region in *I. echinospora* only a single imperfect central tracheid surrounded by a sheath of parenchyma (*figs. 9, 10*). Occasionally in *I. echinospora*, and usually in *I. Englemanni*, two, sometimes three, other such groups can be traced up the leaf.

The phloem is best represented in the chlorophyll-bearing portion of the leaf. It there consists of two strap-shaped bands on the dorsal side, more or less united by their edges, so as partly to surround the xylem. In less distinct form the phloem may be traced downwards to the region of the central bundle.

The development of the ligule was accurately described by (Hofmeister 1), and also by Hegelmaier (1). The latter refers its origin to more than one cell. Since the former gives few figures, however, and the latter none, I shall again briefly outline the course of growth and illustrate it with a few drawings. The ligule originates from a single large vesicular cell protruding from the ventral face of the leaf rudiment. Provision for its rapid growth is shown in the large size of the nucleus of this cell, and the density of the cytoplasm (*figs. 12, 13*). The first division is always parallel to the face of the leaf (*figs. 14, 15*), and usually the second division is parallel to the first. The ligule of *I. lacustris* is described as passing through a filamentous stage; but in *I. echinospora* and *I. Engelmanni* it is hardly worth while to distinguish such a stage, for the filament never consists of more than three cells. The terminal cell then divides in a vertical plane at right angles to the first wall (*figs. 16, 18*). Other vertical divisions follow until the ligule has become a plate of cells of very regular arrangement. *Figs. 18*

and *19* are median sections of the ligule made tangentially to the face of the leaf. Longitudinal sections are shown in *figs. 26, 27, 28, 33, 35*. Growth in length and breadth continues very rapid, and the ligule soon overtops the leaf (*fig. 8*). For some time it remains a single layer of cells in thickness, but eventually it becomes double throughout most of its extent. The doubling begins in the middle region near the base and extends in all directions, never reaching the apex or margin however, which remain to the last but one layer in thickness (*fig. 21*). The expanded part soon reaches its maximum growth. Not so the foot region; this becomes quite massive and deeply embedded in the tissue of the leaf, especially at the sides which grow upward and downward into two prominent cornua. *Figs. 22–25* may help to explain the form of the base of the ligule. *Fig. 25* is a transverse section of the leaf cutting across the cornua above the main place of union of the ligular and leaf tissues. Sections below it show the cornua connected by a transverse band embedded in the leaf; and sections still lower would show portions of the cornua only. The other figures need no fuller explanation than that accompanying the plates.

Along with the growth of the ligule there comes about a differentiation of the cells composing it. There may be said to be four regions. The base is closely surrounded by a layer of small deeply-staining gland-like cells (*s* in *figs. 22, 38*) which we may call the sheath. It forms a conspicuous layer, everywhere investing the base of the ligule, and becoming continuous with the superficial cells of the leaf. Next to the sheath is an irregular layer or band of large empty cells, the *glossopodium* (*g* in *figs. 22, 38;* see also *figs. 23–25*). The glossopodium appears to form the base of the ligule, but the true base includes the sheath which, as a study of the development shows, is derived from the lowermost cell of the young ligule (*fig. 38*). Above the glossopodium are smaller cells containing protoplasm and forming the greater part of the ligule. The apex and margin of older ligules constitute the fourth region; the cells are

shrunken and contorted, their nuclei broken down, and the cytoplasm disorganized.

A study of the ligule of Isoetes to be complete must be accompanied by a comparative examination of the ligule of Selaginella. With this in view I have studied the origin and growth of the ligule in *S. Martensii* and *S. apus*, and compared my sections with the excellent drawings of Professor Harvey Gibson (2). Professor Farmer (1) has expressed the view that the ligules of Isoetes and Selaginella have little in common except their position and name. I have been led to quite the contrary conclusion, to hold in fact that there is a very close homology between the two. What has appealed most to me, in addition to the position of the organs, is the similarity of the regions of which both are seen to consist. The ligule of Selaginella has a glossopodium of large empty cells, sheathed by a gland-like layer, and shows also two upper regions, one of living and one of disorganizing cells. The two are alike also in the absence of chlorophyll, starch, and intercellular spaces; and both show their embryonic character by passing their maximum of growth before the leaf has reached its greatest functional activity. Differences are to be expected, of course, and are chiefly these: the ligule of Isoetes arises from a single cell, that of Selaginella from a group of cells; and, whereas the ligule of Isoetes is almost from the beginning a conspicuous part of the leaf, that of Selaginella is rather late in making its appearance, no trace of it being discoverable until after the sporangium rudiment is plainly perceptible.

THE SPORANGIUM.

The sporangium has repeatedly been made the object of investigation during the last fifty years. Hofmeister (1) was the first to make a careful study of its origin and development. Though his view that the sporangium can be traced back to a single cell has been discredited by later observers, I hope to show that his error was largely due to his exclusive dependence upon longitudinal sections. Except for his failure to see the true

nature of the sporangium rudiment as a transverse row of cells, his account is surprisingly accurate when the imperfect methods of sectioning and staining of that time are taken into consideration.

According to Hegelmaier (2) and Tschistiakoff (1) the sporogenous tissue is differentiated out of a considerable mass of deep-lying meristem between the epidermis and the vascular bundle.

Goebel (1) agrees substantially with the two preceding authors, but is more explicit in his description. The *Anlage* of the sporangium according to him is a group of cells of the leaf base, chiefly the three upper layers. The outer layer gives rise to the sporangium wall, and the hypodermal layer to the archesporium from which all the spore mother cells, trabeculæ, and tapetum are derived. Goebel's account, as confirmed and restated by Sadebeck (1) in Schenck's *Handbuch der Botanik*, has formed the basis of all the text-book descriptions of the sporangium of Isoetes written since that time.

The latest student in this field is Bower (5), whose description is confirmatory of Goebel's except that he traces the origin of the sporangium to a group of superficial cells. This difference, however, is of the very greatest importance. For whereas the derivation of the archesporium by periclinal divisions of superficial cells is the rule in Pteridophytes, the origin of the sporogenous tissue from a hypodermal layer separated from the beginning from the epidermis is a spermatophyte character. The result of Bower's work then is to put Isoetes in line with other Pteridophytes in respect to the origin of the archesporium.

My own results are in the main confirmatory of Bower's as to the origin of the sporangium, though with variations in minor details which may be due to specific differences (Bower studied *I. lacustris*); but as to the later stages of development, especially of the megasporangium, I cannot make my observations harmonize with any accounts hitherto written.

It will, of course, be apparent, when so many discrepancies appear in the descriptions of different investigators, that the

study must be one which involves considerable technical difficulty. This is attributable (1) to the absence of an elongated axis and internodes and the consequent crowding of the sporophylls, and (2) to the early appearance of the sporangium and the consequent difficulty of distinguishing it from the other meristematic tissues in which it is placed. The kinds of evidence on which I have relied in my interpretations may be stated briefly as follows:

1. Study was made of sporangia whose sporogenous tissue was already distinct and unmistakable. Then by comparisons with successively younger sporophylls the attempt was made to trace the sporangium to its earliest rudiment.

2. A careful comparison was made of sections in the three planes, longitudinal, transverse, and tangential. This involved the waste of a great deal of material. For it will be made clear by a glance at *fig. 4* that sections made longitudinal to the stem could give longitudinal sections of very few young leaves, and oftener than not would fail in this altogether, since the leaves have a spiral arrangement; while, in order to obtain transverse and tangential sections, one must cut obliquely to the stem without possessing any clue by which to determine the proper angle of obliquity.

3. The position of the vascular bundle enables one to determine whether the sections are truly longitudinal, and which of a number of serial longitudinal sections is exactly median. This help is available only after the sporangium is distinctly outlined, and somewhat advanced in development, for in case of very early stages of the sporangium, the vascular bundle has not yet been differentiated.

4. In such early stages one must depend very largely upon the ligule, which in position and outline is so definite, and in manner of growth so regular as to make it of the highest importance in assisting one to orient the sections.

5. The sporogenous tissue is often distinguishable from vegetative tissue by a difference in staining. There are three periods when this difference is most manifest. The superficial

cells which form the earliest rudiment of the sporangium frequently take a distinctive cytoplasmic staining, especially in material fixed in Flemming's solution. It must be confessed that this means of recognizing sporogenous tissue is not so trustworthy as one could wish, for at this period of the leaf's history all the tissues are meristematic, and hence readily susceptible to protoplasmic stains. One who studies the origin of sporangia in Lycopodium or Selaginella meets with the same difficulty in those plants, a difficulty in my experience quite as great in these cases as in Isoetes. When the superficial layer of the sporangium has assumed its character as an epidermis, the deeper lying sporogenous cells are easily distinguishable by stain reactions from the surrounding tissues. At a later period the spore mother cells selected out of the general internal mass of the sporangium become quite distinct on account of their denser contents and more intense staining.

Longitudinal sections of young leaves show no space between the base of the ligule and the stem. At this time there is still an active uplifting of cells above the general stem level, a continuance of the process by which the leaf first emerged. When the ligule has grown sufficiently to contain eight or ten cells in longitudinal section the space below it is occupied by one large cell with dense cytoplasmic contents (*fig. 26*). The next change which takes place is a transverse division of this cell as shown in *figs. 27, 28*. Comparisons of successive serial sections show that the two cells shaded in *fig. 27* form the middle of a group of cells arranged transversely to the leaf. This group of cells, distinguishable in good preparations by their deeper staining and larger nuclei, constitute the rudiment of the sporangium. In order to learn its extent and arrangement recourse must be had to transverse and tangential sections.

Most transverse sections of this early stage of the sporangium show that it is five cells in width. Whether or not these can be traced back to a still smaller number I am in doubt. *Fig. 29* certainly shows an example where the transverse row consists of only three cells, and it is clear that the shaded cells of *fig. 30*

may have had their origin in three similar to those of *fig. 29*. But I have succeeded in getting only two such cases as that of *fig. 29*, one in *I. echinospora* and one in *I. Engelmanni*, and have failed altogether to obtain a tangential view.

Tangential sections of the leaf at this early stage are almost uninterpretable. The face of the leaf is so closely pressed against the back of the next younger one that it is quite impossible in most instances to distinguish the tissues of the two leaves or to determine what is a truly tangential section. That shown in *fig. 31* was such as to admit of certain interpretation. The shaded cells occupy the surface of the leaf and clearly correspond to the group which we have already examined in longitudinal and transverse sections. It is probable that another cell seen in the adjacent section to the left of those figured belongs to the same group, making the total number of cells seven.

It is evident from a comparison of my *figs. 26–28* with *figs. 104–106* of Professor Bower's plates, that the longitudinal growth of the leaf base of *I. lacustris* is much more rapid than that of *I. echinospora;* and his figures though not his text suggest that the six superficial cells which make up the sporangium *Anlage* are derived from not more than three rows and probably from but two. If this suggestion be correct, it would bring Bower's and Hofmeister's accounts, so far as regards longitudinal sections, into harmony with each other, and with the foregoing account of *I. echinospora*.

The young sporangium, situated as it is on the hollow side of the leaf crescent, projects little if at all from the surface. By its rapid growth, however, it soon forms an oval prominence at first wider than long, then nearly circular in surface view, and finally considerably longer than wide. In its development I have not been able to establish any regular order of sequence. Starting from such a beginning as figured in *fig. 26*, it is certain that transverse and longitudinal divisions are the first to occur. Then periclinal walls appear (*fig. 30*). The middle cells of the sporangium rudiment are at first most active in dividing, not only in respect to surface growth, but in periclinal divisions also.

Sections adjacent to that represented in *fig. 31* show three or four hypodermal cells which have been cut off from the middle cells of the group and evidently belong to the same series.

There is at no time a single complete hypodermal layer which may properly be termed an archesporium. For when the middle cells have just completed their periclinal divisions the lateral cells are still undivided, and by the time the lateral cells have undergone their first periclinal division the middle of the sporangium is at least three layers deep. A very good example of this is seen in *fig. 42*, which represents the side of quite a large sporangium.

The growth of the sporangium is carried on most actively by the two or three outer layers of cells, as is evidenced by their large size and deeper staining, and the frequency with which they are found in karyokinesis. The divisions of the superficial layer are by no means limited to those in anticlinal planes, as is usually the case with the external cells of sporangia, but for a long time they continue to add to the inner mass by periclinal divisions. In the sporangium of which *fig. 39* shows a section, as many as eight or ten of the external cells were in the act of periclinal division. Even in so old a sporangium as that shown in *fig. 43* the same process is still in continuance. The cells marked with a cross have evidently been derived from the external layer. Though in older sporangia the additions so made go to form part of the sporangium wall, there can be no question that in the younger sporangia they add to the true sporogenous tissue. The bearing of this fact upon the question of what constitutes an archesporium will be considered further on.

It seems necessary to digress at this point in order to make clear some features in which the preceding account differs from what has been recorded by previous observers. Both Hegelmaier (2) and Tschistiokoff (1) assert that the wall of the sporangium is from the beginning ("von Anfang an gesondert") separated from the inner complex, and emphasize with great distinctness the deep-seated origin of the sporogenous tissue.

Goebel (1) states his approval of Hegelmaier's view, but the occasional periclinal division of the external cells does not escape his notice, though he considers it as merely adding to the thickness of the wall. Bower (5), on the other hand, observed both the superficial origin of the sporangium and the failure of the first periclinal divisions to completely delimit the archesporium.

As already stated, I do not find the outer wall separate from the sporogenous complex from the beginning. On the contrary, it is distinctly active in increasing the dimensions of the sporangium. Ultimately the superficial layer loses some of its protoplasmic contents, and assumes the appearance of an epidermis. It sometimes happens that this separation of a wall layer occurs quite early (*fig. 41*), but oftener it is not till the sporangium has come to consist of many hundred cells. Even then periclinal divisions do not entirely cease.

According to my observations there is no regularity in the arrangement of the cells within the sporangium. The discovery of this was a great surprise to me, for Goebel's statement is very explicit: "Each of the cells composing the archesporium has an independent growth," and in this he has been corroborated by Sadebeck and Farmer. Bower has not traced the history of the sporangium with any fullness; he merely states that his results are confirmatory of Goebel's and his figures certainly convey the impression that each cell of the archesporium has an independent growth. But he has made use of the same style of drawing in representing the sporangia of other genera (Lycopodium, Selaginella, Equisetum), in which no such claim is made. In view of my own observations and of Bower's drawings, it is difficult to know just how much is meant by the phrase "independent growth."

In the case of bryophyte antheridia the primary spermatogenous cells are clearly distinguishable throughout the whole development of the antheridium, although each may become divided up into a hundred or more sperm mother cells. The individuality of the original cells is marked in several ways:

their outer walls remain straight and become thicker than those which subsequently appear within them; and the incomplete separation of the derivatives of any single primary sperm cell from one another and their complete separation from those of other primary cells are shown by their dividing concurrently. I have frequently observed in the antheridia of Polytrichum, Porella, Marchantia, and Asterella that all the cells derived from one of the primary sperm cells enter into karyokinesis together, finish their division, and enter into the resting condition together, quite independently of what may be going on in the derivatives of other primary cells. In such cases it is quite proper to speak of an independent growth; for the separation and isolation of each group by thickened walls are sufficient to insure a simultaneous exposure and obedience of all the cells to the physiological stimulus which induces karyokinesis.

Are there any indications of such independent growth in the sporangium of Isoetes? I can find none, either in the arrangement of the tissues or in the presence of thickened walls which mark the boundaries of the original archesporial cells, or in the simultaneous entrance of the cells of each group into the phases of division. All the mature cell walls of a growing sporangium are of equal thickness; and in marked contrast to what is seen in the leaves there is no regularity of stratification or lining-up of the cells. I am forced to conclude that the sporangium of Isoetes (at least of *I. echinospora* and *I. Engelmanni*), just as the microsporangium of angiosperms, grows as a unit and not as a number of individual segments.

Before continuing the subject of the development of the sporangium it will be convenient to consider the formation of the velum. The velum makes its appearance very early in the history of the sporangium, almost as soon in fact as the first periclinal divisions of the superficial cells. It is formed immediately below the ligule. Hofmeister (1) says: "Of the two cells into which by a transverse septum the cell underneath the place of insertion of the ligule is divided the upper one becomes the primary cell of the velum and the lower the primary mother cell

of the sporangium." It has already been said that Hofmeister was in error because of failure to notice the lateral extension of the sporangium rudiment. Even allowing for this, however, I am not able to agree fully with his account. It appears rather that the upper tier of cells while giving rise to the velum makes some additions at the same time to the sporangium. In other words, the velum is a sterilized portion of the sporangium. Some sections seem to admit of this interpretation only, though others, such as *fig. 32*, are not unfavorable to the view that the separation of velum and sporangium proper is accomplished by the first transverse division of the sporangium rudiment.

Early stages of the velum may be seen in *figs. 32–36;* it is at this time a transverse row of slightly projecting cells. Its cells soon become comparatively empty, contrasting strongly with the young sporangium. Growth is very rapid and in an upward oblique direction; in some cases there is a tendency to a downward growth also, such as obtains among terrestrial species.

The velum reaches its full size much sooner than the sporangium, and is not affected by the changes which determine the character of the latter. The cells of the interior become large and lose their contents; those of the inner surface layer — that adjacent to the sporangium — are smaller and more regular in size and outline, and have a semi-glandular appearance. In many species of Isoetes many of the cell walls of the velum and of the leaf region adjoining the ligule become lignified and take on spiral and annular thickenings. *I. echinospora* and *I. Engelmanni* offer no exception in this respect, the thickenings being much more pronounced in the latter species. The change first appears in proximity to the ligule, and spreads thence into the remoter parts of the velum and of the leaf. The thickened cells never have any connection with the vascular bundle (*figs. 23, 24*).

FURTHER DEVELOPMENT OF THE MICROSPORANGIUM.

In origin the two kinds of sporangia are identical, and for a considerable period of their development they exhibit no

observable difference. The general statement of the text-books, following Goebel and Sadebeck, is that they follow the same course of development only so far as the formation of the archesporium, and thereafter may be distinguished by their manner of growth. It is said that in the megasporangium certain archesporial cells divide only by periclinal walls, but in the microsporangium all the archesporial cells divide both anticlinally and periclinally, and that in this respect the two are distinguishable from the archesporial stage on. Such is not the case in the forms which I have studied. In these all the archesporial cells, whether of megasporangium or microsporangium, undergo divisions in all directions, and the similarity of the two kinds of sporangia continues much beyond the archesporial stage. Not only do they agree in origin, but up to a time when they are eight or ten cells deep, they agree absolutely in manner of growth, and exhibit no histological features by which one may determine whether a given sporangium will bear microspores or megaspores.

As an example, consider the sporangium of which *fig. 43* represents a section. It had advanced so far beyond the archesporial stage as to contain about 8000 cells. From the position of its sporophyll we may infer it was destined to become a megasporangium. But there is nothing in the arrangement or character of the cells or in their mode of division to warrant that prediction, or to enable us to say such a group of cells will become a trabecula, and such a group will produce spores. It has the characters neither of a megasporangium nor of a microsporangium, but is as yet quite undifferentiated.

The first changes which occur to mark the microsporangium are those which lead to the differentiation of the spore mother cells from the trabeculæ, sporangium wall, and tapetum. Previously there has been no essential difference in the cells as to size, form, or contents, excepting the external layer. But when the sporangium is approaching a limit of cell multiplication, that is, when the number of cells is 15,000–20,000, certain regions begin to lose their power of division and reaction to stains, while

other regions become more active in division and more deeply stainable. The former may be called the sterile regions, since they form the walls, trabeculæ, and tapetum, and the latter the fertile region, since they give rise to the spores. Even in unstained sections the difference is noticeable as one of relative abundance of protoplasmic contents.

At first it is difficult to see clearly the limits of the regions or to make out their arrangement. But in older sporangia they are seen to be disposed in irregular bands extending from the base of the sporangium outwards to the wall. The published drawings, and unfortunately in some cases the written description also, are calculated to convey an erroneous idea of the trabeculæ. They are not partitions, but, though irregular in outline and frequently branched and anastomosed, are comparable rather to pillars. It is accordingly incorrect to speak of the sporangium as chambered, for the fertile cells are not segregated into loculi, but form a continuous mass pierced here and there by the trabeculæ. It is hoped that *figs. 44–47* will make the relations of the trabeculæ clear. The shaded portions of these drawings represent the fertile regions, and the unshaded portions the trabeculæ and walls. The continuity of the sporogenous mass is clearly seen in the tangential section (*fig. 46*).

A more detailed account of the development of the microsporangium will now be given. *Fig. 48* shows a small portion of a microsporangium in which the differentiation into sterile and fertile regions has just begun. The fertile cells stain deeply and are still rapidly multiplying, as is evidenced by the many karyokinetic figures. The sterile cells have almost entirely ceased divisions, though here and there a dividing cell may be found. It is important to notice that the one character in which the two regions differ is in the relative abundance of protoplasm, the fertile cells being densely filled with deeply staining cytoplasm, while the cytoplasm of the sterile cells is beautifully vacuolated. In all other respects the cells of the two regions are essentially alike. They are not markedly different in size,

or in the size and appearance of their nuclei, nor is there anything in their arrangement to suggest a difference in their origin or growth. In fact, as Professor Bower has pointed out, there is here a most excellent illustration of the sterilization of sporogenous tissue.

The trabeculæ at this age show about 15–25 cells in cross-section (tangential section of the sporangium), and are more or less cylindrical. There is as yet no tapetum. Towards the outer and inner sides of the sporangium the trabeculæ are continuous with about three layers of cells which form the sporangium wall (*fig. 49*). That the trabeculæ and walls are of the same nature, both being the result of sterilization of potentially sporogenous tissue, is proved not only by the similarity of their cells, and their passing uninterruptedly into one another, but also by their relation to the tapetum, which is formed out of the layer that lies next to the spore mother cells.

The inner cells of the trabeculæ, those which become the trabeculæ proper (*i. e.*, exclusive of the tapetum), are at first isodiametric and in no way different from the outer ones. But while the latter are undergoing a transformation into tapetum, the former undergo changes which are dependent on the growth of the sporangium. As the dimensions of the sporangium increase — a change which goes on rapidly at the period when the sporogenous cells are multiplying — the trabeculæ are necessarily lengthened. This is accomplished, not by division of the cells, but merely by their elongation. At the same time they suffer a lateral compression from the growing sporogenous cells and become flattened (*fig. 50*). The tabular form of the cells doubtless furnishes the ground for the common view, which ascribes the form of the cells to the direction of their division planes. Such a view is incorrect, however, for divisions have entirely ceased in this region before the elongated form of the cells is attained. The shape of the cells is easily accounted for by their growth in the one direction possible for them while yielding to the pressure of the turgescent mother cells.

In this connection it may be remarked that with the possible exception of the tapetum all the cells of the sporangium, after

losing their power of division, enter upon a period of growth which is quite comparable to that occurring in vegetative meristems. The difference in size of the sporangia represented by *figs. 43* and *63*, which are drawn under the same magnification, is due partly, it is true, to increase of the number of cells, but a glance at the two figures shows there has been also a decided growth of the individual cells.

Accompanying the modification of the trabecular cells, there is a change of form of their nuclei. These become first elongated and oval (*fig. 50*), and finally spindle-shaped, suggestive of the changes which attend the development of the vascular strand out of the tissues of a growing point. Instances of much greater elongation than that shown in *fig. 51* are frequently met with, though in other cases the changes are comparatively slight. The nuclei at this time are relatively large and prominent, and appear to form the center of aggregation of what little cytoplasm still remains in the trabecular cells. In old sporangia the cells of the trabeculæ are nearly or quite empty, and much compressed.

Bower has discussed the function of the trabeculæ. They may serve for mechanical support of the sporangium, or to afford a larger nutritive surface, or, since the two functions are not incompatible, for both. The relation of the trabeculæ to the base of the sporangium where it is closest to the vascular bundle, and the resemblance of the nuclei to those of plerome regions in general, suggested to me that the trabeculæ might be the channels through which nutriment is supplied to the spores; but the suggestion is not borne out by observation. It is clear that in a hydrophytic plant no elaborate apparatus is needed to provide the sporangium with water, which can easily enter directly from the outside; and an examination of my sections shows that the organized food stuffs, such as starch and oil, pass to the spores through the inner wall of the sporangium, and not through the trabeculæ.

The tapetum, as already stated, is organized out of that layer of the sterile cells, whether of wall or trabeculæ, which is in contact with the fertile cells. At a stage between those shown in *figs. 48*

and *50*, the cells of this layer multiply rapidly. They are frequently found in mitotic division, with the axis of the spindle always perpendicular to the surface of the trabeculæ or sporangium wall. Divisions may still go on here after the spore mother cells have reached maturity, and the changes of the trabeculæ are nearly complete. In this way the tapetal cells become very numerous, but reduced in size. They form but a single layer except in limited areas, where a doubling may sometimes occur.

At first the tapetum is not deeply stained (*fig. 50*), but as the spore mother cells prepare for their tetrad division, the tapetal contents increase in density, and they continue to do so until they surpass young spores in this respect.

From what has been said, and from *figs. 47*, etc., it will be understood that the tapetum completely invests the trabeculæ and sporangium wall, forming a lining layer everywhere between the spore mother cells and the sterile regions. It is a persistent layer, and in this respect is to be contrasted with that of most ferns and angiosperms. In these latter the walls of the tapetum break down and are dissolved, the cells become disorganized, and their materials, mingling with the other contents of the sporangium, are used to nourish the mother cells or young spores. In Isoetes, however, as in Lycopodium and Selaginella, no such disorganization of the tapetum occurs. Its cells do not fall apart and its walls are not absorbed. In old sporangia it is still recognizable, though often its contents have been lost and the walls are pushed nearly together.

Probably the tapetum can best be regarded as a gland or layer of glandular cells. If so, the manner of action in a persistent tapetum, such as that of Isoetes, Lycopodium, and Selaginella, must be quite different from what it is in a tapetum which is regularly disorganized and absorbed. In the one case the nutrient substances secreted by the cells must be passed on through the walls into the cavity in which the young cells are growing. In the other case there can be little or no passing of nutrient substances through the walls, but at the proper time the

secreted materials are rendered available by the total collapse of the cells.

In many plants also, especially in those in which the tapetum undergoes complete disorganization, it is common for the tapetal cells to become multinucleate, the division of the nuclei being sometimes accomplished by karyokinesis, but mostly by amitosis. The cells of the tapetum of Isoetes, in this respect again agreeing with Lycopodium and Selaginella, are uniformly uninucleate.

In almost every sporangium examined the number of layers of cells outside the fertile regions when they first become distinct is three. In a very few cases there were four layers. As already shown, the innermost of these becomes tapetum. Of the other two layers, one, apparently the hypodermal, usually undergoes division, so that the wall region ultimately consists of three layers outside the tapetum.

At the base of the sporangium, between it and the vascular bundle, are a few layers of cells which may be regarded as the inner wall of the sporangium. The exact origin of these I have not been able to make out. Whether, like the outer wall, they are derived from the sterilization of sporogenous tissue, or whether they are derived from the tissues underlying the original archesporium, I cannot say. It is always difficult in all sporangia except the very youngest to define the exact inner limits. Between the vascular bundle and the three or four outer layers where growth and division are most actively carried on, there is a mass of small cells staining deeply. Such a section as *fig. 38* makes it probable that all the tissues between the parenchymatous sheath of the xylem and the outside arises from the sporangium *Anlage*, and that therefore the inner wall arises also by sterilization.

The formation of the microspores in Isoetes takes place in much the same way as in other vascular plants. After the fertile regions have ceased their cell divisions, the cells and their nuclei pass through a period of rest and enlargement. The nuclei especially increase in size and become rich in chromatin. At the same time the cytoplasm remains dense and never

shows the vacuolated appearance of the sterile cells. Shortly afterwards the mother cells break away from the tapetum, which from this time on gains in density and apparent activity. The mother cells, at first in a continuous mass, soon break up into smaller and smaller groups of cells by the enlargement of the cavity in which they float. Finally the individual cells fall apart and round up, and pass rapidly through the two divisions by which the microspores are formed.

No attempt was made to follow closely the cytology of these divisions because it was found impossible to make any satisfactory observations on the corresponding divisions of the megaspore mother cells. The following notes may however be of interest. The achromatic figures appear to have a polycentric origin, and the chromatin passes through a synapsis stage. All the nuclei make their preparation for division and begin to divide almost simultaneously, and this notwithstanding their immense number. It is possible to find a better series of karyokinetic figures in a single sporangium of many ferns, where there are but sixteen mother cells, than in an Isoetes microsporangium where the mother cells number three or four times as many thousand. This I think may be regarded as an additional proof of the growth of the sporangium as a unit, and not as an aggregation of segments.

In the majority of cases the two divisions are of the type which is characteristic of cycads and monocotyledons, and has been called "successive;" that is, the first division of the nucleus is followed by the formation of a cell wall before the immediately following division of the daughter nuclei (*fig. 53*). The spores in this case are bilateral and may have their nuclei in one plane or in two planes at right angles to each other. But it is not at all infrequent to find the divisions of the simultaneous type; that is, the first division of the nucleus is not attended by cell division, but before a wall is formed between the daughter cells each new nucleus begins its second division (*fig. 54*). In this case the spores may be of the bilateral type, as in *fig. 55 a* and *b*, or they may be tetrahedral as in *fig. 55 c*. Much

diversity may be found within a single sporangium. *Figs. 53 a, b, c,* and *54 a, b, c,* were all taken from the same section of the same sporangium. Probably the variation in this respect is not of great importance except as indicating that the divisions of Isoetes have not acquired so definite and settled a character as those of most other plants.

Although the nuclei of the young spores may arrange themselves in typical tetrahedral fashion, there is an important difference between their relation here and in the tetrahedral divisions of dicotyledons, Lycopodium, etc. In these it is well known that all four nuclei (of such a stage as *fig. 54*) become connected by spindle fibers, and that the walls separating the spores are formed in connection with the thickening of the cell plates of the six spindles. In spite of careful search I have been unable to find in Isoetes any such sextuple spindles. The daughter nuclei are connected only in pairs, as in *fig. 53* or *54*. In what way the spore walls originate in such cases I cannot conjecture. It seems certain they are not formed in connection with the achromatic figures, unless it is possible that the cell plate, which is always present in the first division, may make its influence felt later on, and ultimately serve as the basis of the wall.

The young tetrads soon fall apart, and the individual spores lose their angularity and round up, still retaining traces, however, of the bilateral shape impressed upon them by their manner of origin. When once the permanent form is assumed there is little further increase of size. The mature spores of *fig. 56* are little larger, it will be seen, than the newly formed spores of *fig. 51*.

An interesting phenomenon in connection with the microspores is the extreme smallness of their nuclei in comparison with those of the mother cells. One would naturally expect the relative volumes to be about 1 : 4, or the relative diameters to be about 3 : 5 (since $\sqrt[3]{\frac{1}{4}} = \frac{3}{5}$ nearly). But the volume of the microspore nucleus is really no more than one twelfth of this estimate; or to express the comparison in another way, it would need the nuclei of fifty microspores combined to equal the

volume of one mother cell nucleus. Very likely similar reductions in the volume of the microspore nuclei occur during the tetrad division of other plants, but I have not seen any other case where the disparity of size is so great, nor do I remember to have read any record of such a reduction.

The number of spores formed within a microsporangium is enormous — much greater than in any other living plant. In some species it is said to exceed a million. But the largest number I have found in *I. echinospora* is 300,000. My estimates place the average number from 150,000 to 250,000.

As is well known, no provision is made for the dehiscence of the sporangium wall. The spores are set free only by the decay of the tissues enclosing them.

FURTHER DEVELOPMENT OF THE MEGASPORANGIUM.

My observations on the development of the megasporangium differ very much from those of previous investigators, so very much, indeed, that I would be loath to present them at all had I not confirmed them again and again by long and careful study. These differences are concerned not only with the origin of the archesporium and early growth of the sporangium, which have been already spoken of, but they involve also the manner of selection of the mother cells and the origin and behavior of the tapetum. A discussion of the points at issue will be reserved until the general history of the megasporangium has been considered.

One of the first megasporangia which I sectioned presented the appearance shown diagrammatically in *fig. 67*. The two large cells *M* and *M* are evidently megaspore mother cells, but what is the group of cells *a*, corresponding to them in outline and position? It consists of six cells in all, three in the section under examination, and three others in the adjacent section. A little search discovered other similar groups of a variable number of cells, sometimes but two or three, often five or six. If the number had been constantly four the groups might have been regarded as spores resulting from a precocious division of

the mother cells. But that explanation being precluded it became necessary to determine their relation to the single large mother cells, and to learn their later and earlier history. In attempting to do so I have become convinced that a very large number of cells are potentially megaspore mother cells, that a considerable number of these make a start to differentiate themselves fully from the sterile cells, but that comparatively few are finally successful in reaching the large size and well-nourished condition necessary for the production of megaspores.

The changes which first distinguish the megasporangium occur relatively earlier than those which mark the microsporangium. In the latter, as we have seen, the first change is the separation of certain sterile regions from the fertile cells as indicated by a difference in cell contents. In the former, however, changes occur at a considerable time before there is any possibility of distinguishing the trabeculæ. When the megasporangium has reached a stage of development considerably more advanced than that shown in *fig. 43*, a change is discernible in many of the cells which form the third and fourth layers approximately. The whole sporangium has at this time entered upon the period of enlargement due to the growth of the individual cells. But in *fig. 63* it is clear that certain cells have greatly outgrown their fellows. Their well-nourished condition is attested by the density of their cytoplasm and their large nuclei, which contain many nucleoli. All these enlarged cells are engaged in the struggle to become mother cells. Which and how many will be successful will probably depend upon their holding an advantageous position with respect to the supply of nutriment, perhaps also to their having obtained an earlier start.

It does not always happen that a considerable group of cells enlarge together. Indeed, it is a comparatively rare case when all the cells of the third and fourth layers enlarge to any considerable extent. Sometimes the enlarging cells are in more or less isolated groups separated by cells of ordinary size. *Fig. 64* shows such a group of cells, taken from the side of a sporangium.

Quite often, too, it happens that one cell gets the advantage almost from the beginning. But it may be stated as the rule that there is a selection and partial enlargement of many more cells than can ultimately become mother cells, and these enlarging cells belong mostly to the third and fourth layers of the sporangium, either extending continuously across the sporangium or occurring in groups separated by ordinary cells. That this condition is associated with the selection of megaspore mother cells is proved, I think, by the fact that enlarging cells, comparable to those of *fig. 63*, are never found in the sporangia formed late in the season, that is, in those which are to bear microspores.

What becomes of the defeated cells? This is a difficult question to answer, for since there is so much variation in the early condition of the megasporangium it is impossible when examining one of the later stages to tell just what the antecedent conditions in that sporangium may have been. From the frequency with which karyokinetic figures appear in the cells surrounding the nearly mature megaspore mother cells, it seems pretty certain that the cells which have been left behind in the struggle simply divide until their products have the general size and appearance of the other cells of the sporangium. If the enlargement has not gone very far the cells retain their angular configuration; if it has gone further the cells may round up while exerting a considerable pressure on those adjacent. So I interpret the group *c* in *fig. 67*.

Fig. 66 will furnish a good illustration of the behavior of the unsuccessful mother cells, although no single section can be so convincing as a series of them. The tissues are somewhat contracted, but this defect does not hide the rounded form of certain groups of cells, and their marked resemblance, except in being multicellular, to the mother cells. The section contains but one fertile mother cell, the one labeled *m*. One other is situated in the opposite end of the sporangium, just beyond the limit of the figure. The cell *a* is undergoing division, the mitotic figure being seen in the adjacent section. An interesting fact

which goes far to explain the division of the groups *b*, *b*, is the occurrence in adjacent sections of larger undivided cells (fertile mother cells), similar to *m*, and so situated as to be almost or quite in contact with the dividing groups. Their proximity accounts for the failure of the groups *b*, *b*, to produce spores. Some of the smaller and less rounded groups probably represent mother cells which suffered an early defeat, while the larger groups represent those which held out almost to the last. Such cases as these, which can be easily duplicated in rapidly growing sporangia of the right age, are conclusive, it seems to me, when considered in conjunction with the manner of growth of the sporangium, to show that the fertile mother cells are selected by their advantageous environment and not by any strict morphological position.

The fertile mother cells increase enormously in size before dividing into spores. Their nuclei maintain a proportionate growth, and their cytoplasm remains dense though not homogeneous, and frequently contains grains of starchy matter and drops of oil.

Notwithstanding the large size of the mother cells and of their nuclei I was unable to make any detailed study of their division. About the time when division occurs, the cells seem to be peculiarly liable to suffer plasmolysis, for under the action of the fixing agent they are contracted to a mere fraction of their proper volume. When sectioned in this condition they are seen to lie free in large cavities which presumably they filled completely when living, and they stain so intensely that it is impossible to make out any details of the karyokinetic process. I have not once had the good fortune to see karyokinesis in an uncontracted megaspore mother cell, although the corresponding phase of the microsporangium offers no technical obstructions to cytological study. The liability of the megaspore mother cells to suffer contraction in the process of fixation was noticed by Kienitz-Gerloff (1) and other investigators; it is possibly associated with the entrance of the nuclei into the synapsis stage.

The young megaspores almost invariably have the tetrahedral arrangement, as in *fig. 59*. Occasionally the bilateral arrangement is found, in which case the divisions so far as observed are successive (*figs. 60, 61*).

The further growth of the megaspores, the manner in which their walls are laid down, and the storing of reserve material, were not investigated.

The arrangement and subsequent development of the trabeculæ and tapetum of the megasporangium offer, as is to be expected, a rather close homology to what is seen in the microsporangium. The trabeculæ are formed out of the same kind of cells as compose all the other parts of the young sporangium. I do not discover any grounds for considering them the product of a peculiar kind of growth. They are altogether unrecognizable in the young sporangium, and their position when first outlined seems to be determined by that of the mother cells. Not until these have been selected and considerably enlarged is it possible to distinguish the trabeculæ, which then appear as feebly-staining bands extending from front to back across the sporangium midway between the fertile cells.

The cells of the trabeculæ proper undergo the same process of elongation and flattening, attended by elongation of their nuclei, that has been described as occurring in the microsporangium. The only noticeable difference is that in the megasporangium the trabeculæ are relatively fewer in number and more massive. For example, in one case, an exceptional one, I counted 400 cells in a cross section of a trabecula, whereas in a microsporangium the number of cells in a cross section of a trabecula rarely exceed fifty, and is oftener under twenty-five. This is only another way of saying that the process of sterilization has gone much further in the megasporangium than in the microsporangium. The total mass of the megaspore mother cells in a sporangium is only a small fraction of that of the combined microspore mother cells, though doubtless the total volume of the mature spores in the two cases is about equal.

The tapetum is formed in this case also out of those layers of the sterile cells which border upon the fertile cells. No doubt a considerable part of it is derived from the unsuccessful mother cells; but as these are the homologues of the trabecular cells of the microsporangium, being merely sterile sporogenous cells, the homology of tapetum and trabeculæ in the two sporangia is complete. The only difference which it is necessary to notice is the greater abundance of the tapetum in the megasporangium. Instead of being a single layer it is several layers in thickness (*figs. 57, 58*), and often projects into the sporangial cavity in the form of irregular papillæ, especially from the base of the sporangium. A rounding up of the cells immediately about the megaspore mother cells, such as is described and figured by Goebel, I was never able to find.

Though the megaspore mother cells do not lie in contact with one another as the microspore mother cells do, but are isolated in groups of one or sometimes two, the cavities in which they lie become continuous in the older sporangia. This is brought about by a very great enlargement of the cavities after the formation of the spores. The enlargement seems to be due to turgescence, induced probably by the osmotic activity of the substances surrounding the spores. It cannot be accounted for by mere growth of the wall cells, nor by that of the young spores, for these do not completely fill the cavities. I have computed the enlargement of the megasporangium after all cell divisions have ceased to amount to an increase of three or four times in volume. A similar change of size, though less in extent, occurs in the microsporangium.

If the preceding account of the development of the sporangia, especially of the megasporangia, be compared with the account given by Goebel (1) and Sadebeck (1), it will be seen that the differences are considerable, and of much theoretical importance. According to these writers certain cells of the archesporium divide only by the periclinal walls which serve to cut off the primary tapetal cells. In these no anticlinal divisions occur. One cell of each of the rows formed in this manner,

apparently the innermost one, though that point is not made clear in the descriptions, becomes the megaspore mother cell.[1] In certain other archesporial cells divisions take place in all planes, but more particularly in the anticlinal direction. The products of these latter cells give rise to the trabeculæ. Vines in his text-book gives nearly the same description, but says that the archesporial cell from which the megaspore mother cell arises undergoes but a single division.

If the assertion be correct that certain archesporial cells develop only into trabeculæ and certain others only into mother cells and tapetum, it is clear that there must be two categories of archesporial cells, one set destined to become sterile, the other to become fertile; and these, although indistinguishable in appearance and size, are quite unlike in their mode of division and growth and in the ultimate fate of their derivatives. It is impossible, too, to escape the inference that the megaspore mother cells are already determined in position and number when the sporangium has got no further in its development than to the differentiation of an archesporium. Further, the sporangium must be regarded as compound, each fertile archesporial cell representing a separate sporangium, and each sterile one an imperfect wall. These conclusions, which I think are logical and necessary deductions from Goebel's description, are all inconsistent with the development of the sporangium as I have found it in *I. echinospora*.

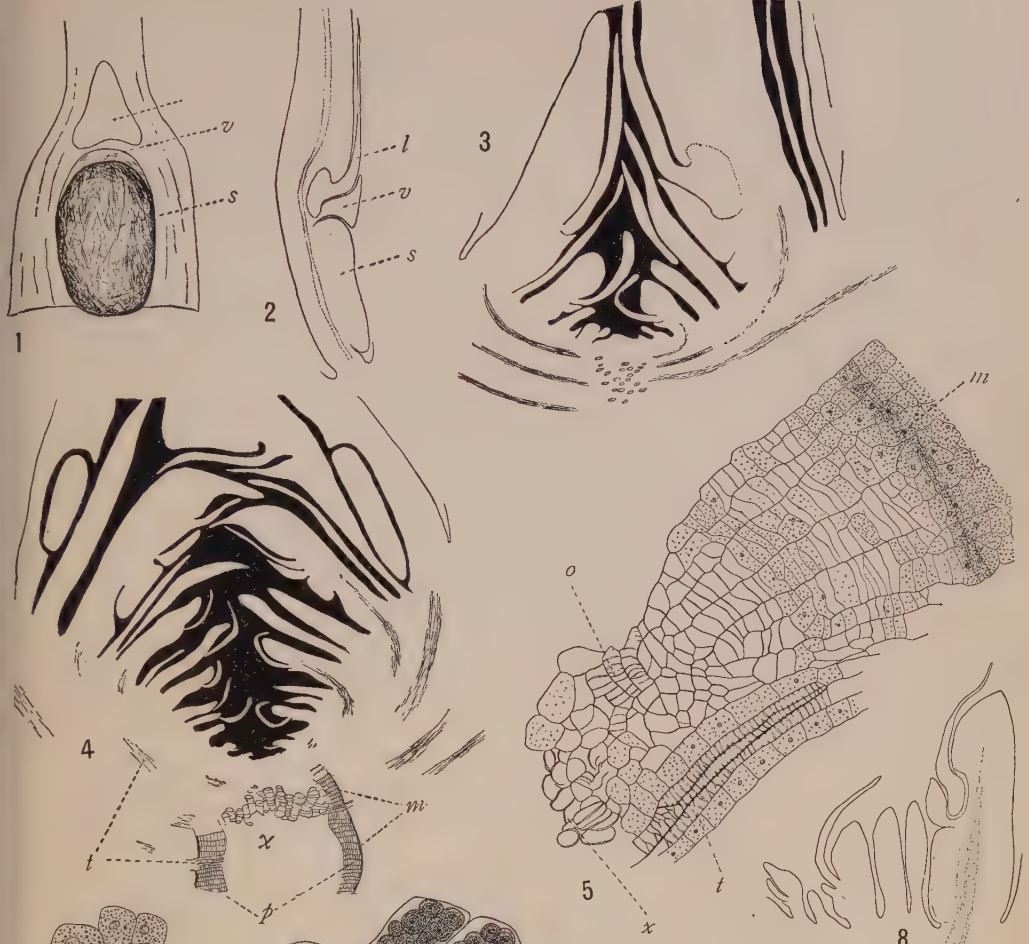
In order to bring out the points of contrast more clearly I will summarize them. I do not find any difference among the archesporial cells either in manner of development or of growth. I find no flattened tapetal cells overlying the megaspore mother cells. I find no grounds whatever for the assertion that each archesporial cell follows an independent growth, or that each megaspore mother cell represents one archesporial cell. I do not even find a single definite hypodermal archesporium which can stand as the starting point of the inferences above enumerated. On the other hand, I find the derivatives of all

[1] See, however, SCHENCK's Handbuch 3 : 392.

the archesporial cells dividing in various planes, and blending indistinguishably. The sporangium is single, not multiple, and the megaspore mother cells are not morphologically predetermined but are physiologically selected from among a large number of potentially sporogenous cells.

Though the certainty of the matter must depend upon observation, it may be pointed out that the number of megaspores has a bearing upon the question. A megasporangium contains from 150 to 250 megaspores. If we take 200 as the average, it represents fifty mother cells, that is, according to the current view, fifty archesporial cells. To this we must add at least fifty others for the trabeculæ, giving a total of one hundred archesporial cells. It does not need a very careful examination of *I. echinospora* to demonstrate the impossibility of there being so large an archesporium, for when the sporangium has a superficies of one hundred cells it is far past the archesporial stage. It is, I think, absolutely certain that each archesporial cell gives rise to several megaspore mother cells, as well as to trabeculæ and tapetum. In the microsporangium, too, the trabeculæ alone outnumber the archesporial cells (*cf. figs. 31, 46*); and their extreme irregularity and frequent branching and anastomosis make their origin each from a single cell exceedingly improbable.

[*To be concluded.*]



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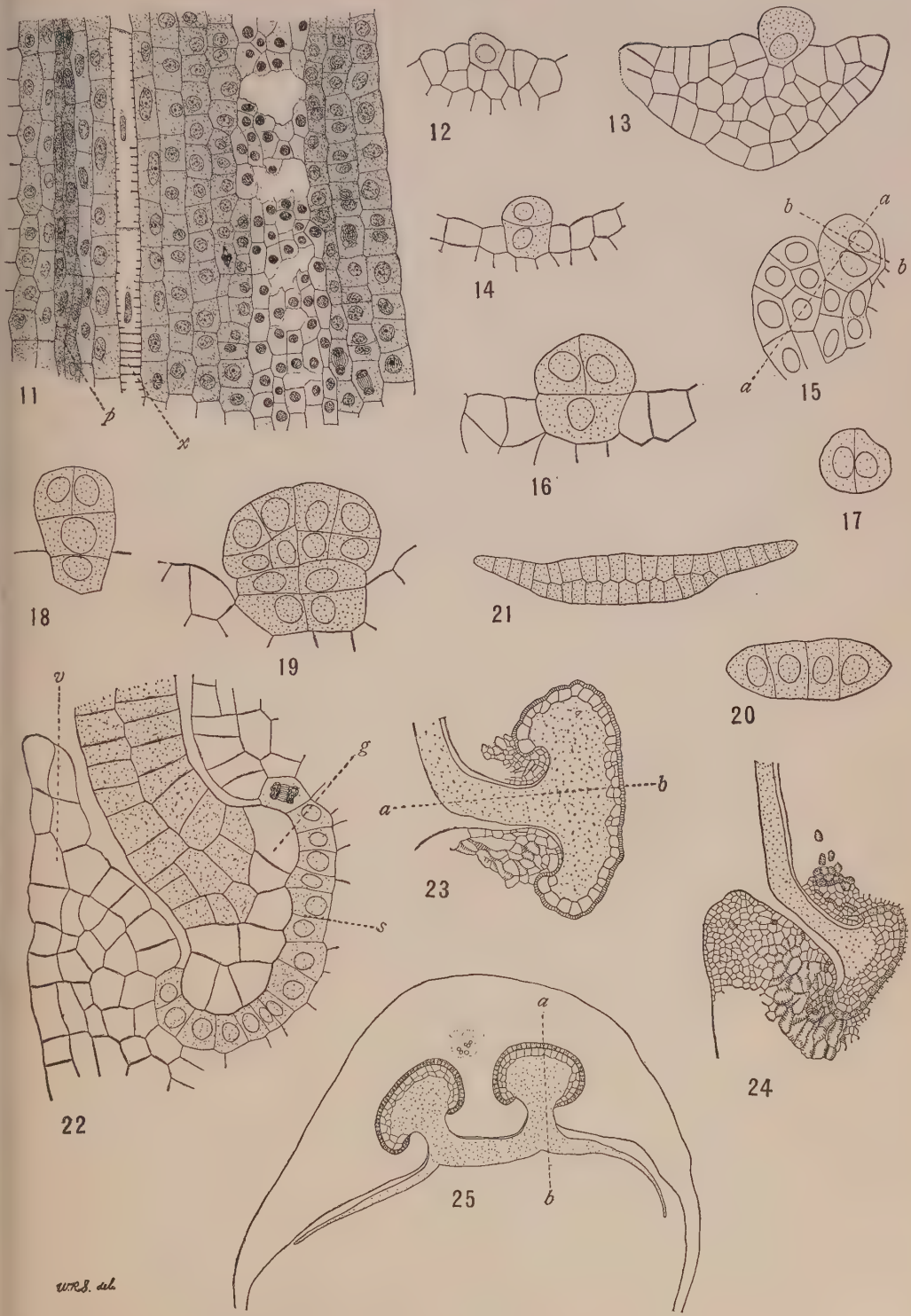

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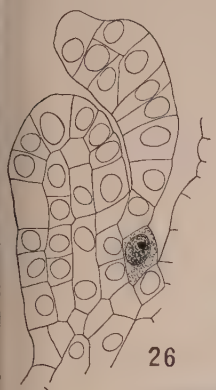
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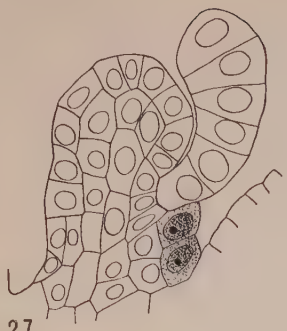
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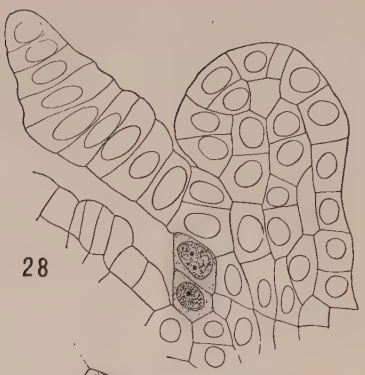
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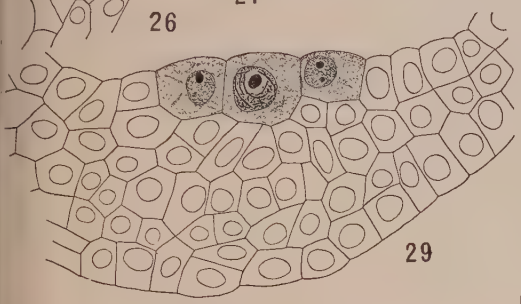
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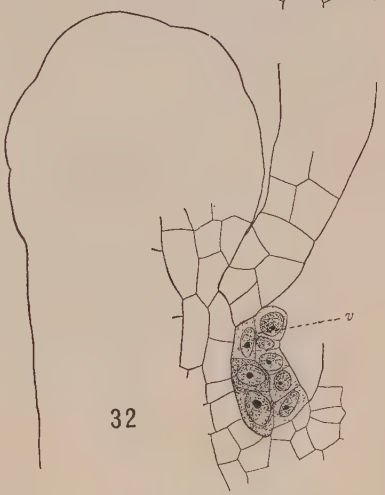

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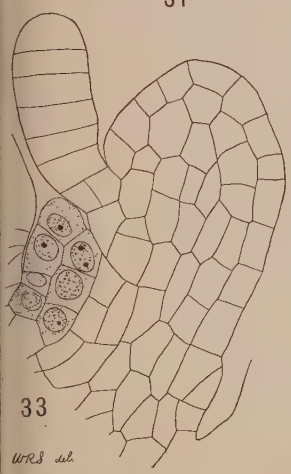
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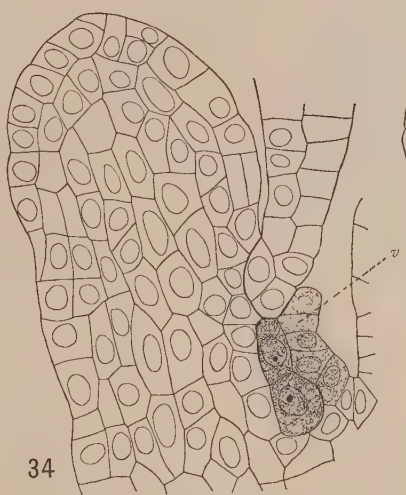

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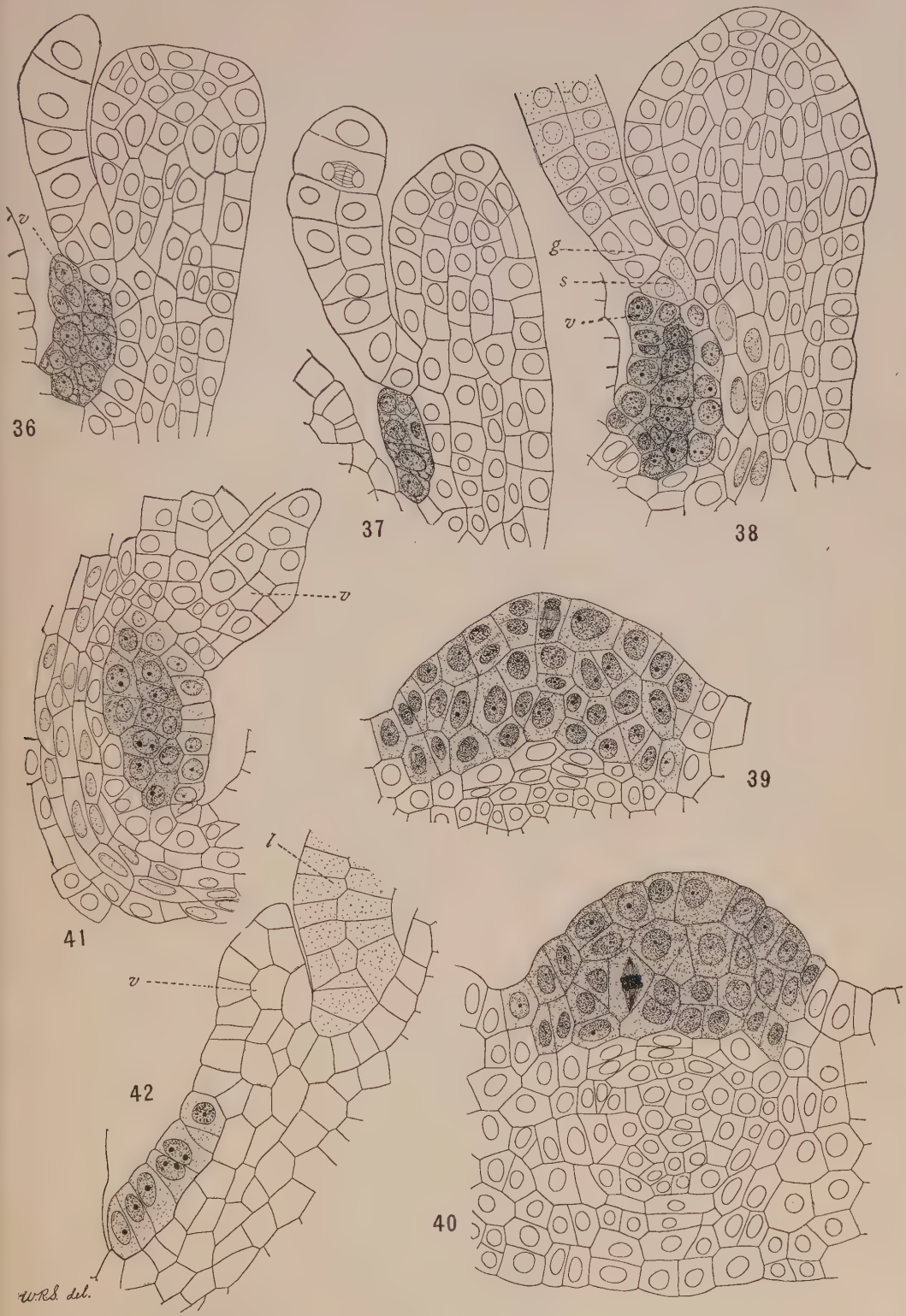

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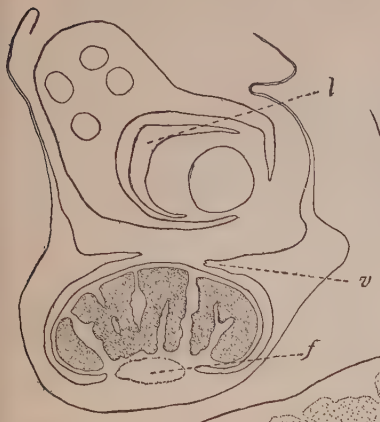

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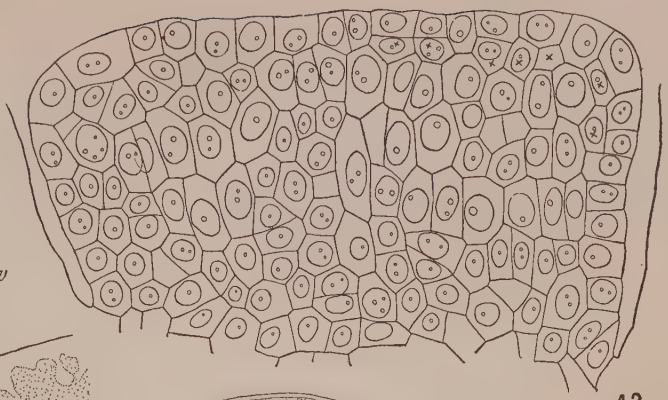

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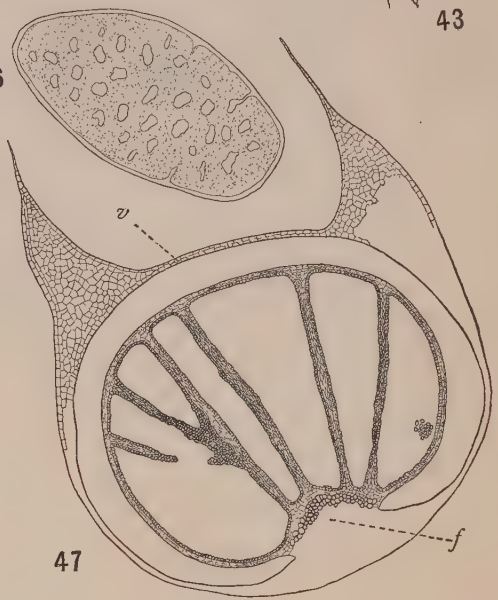

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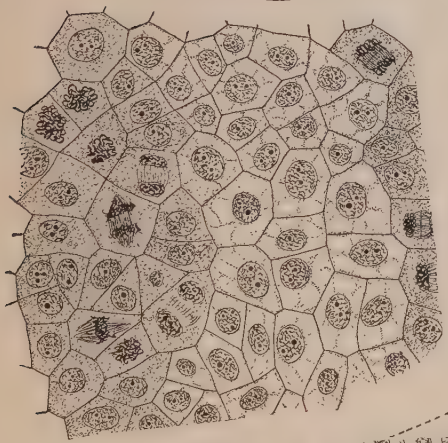

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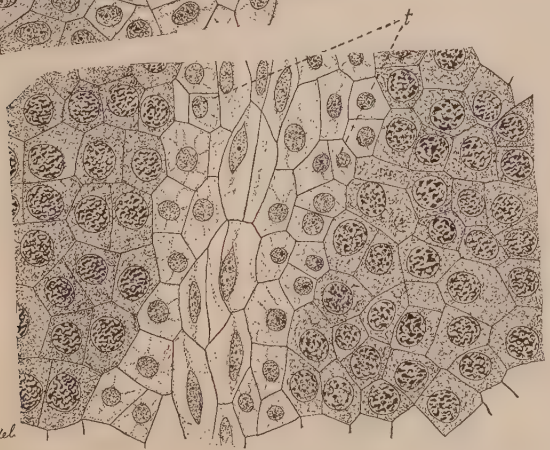

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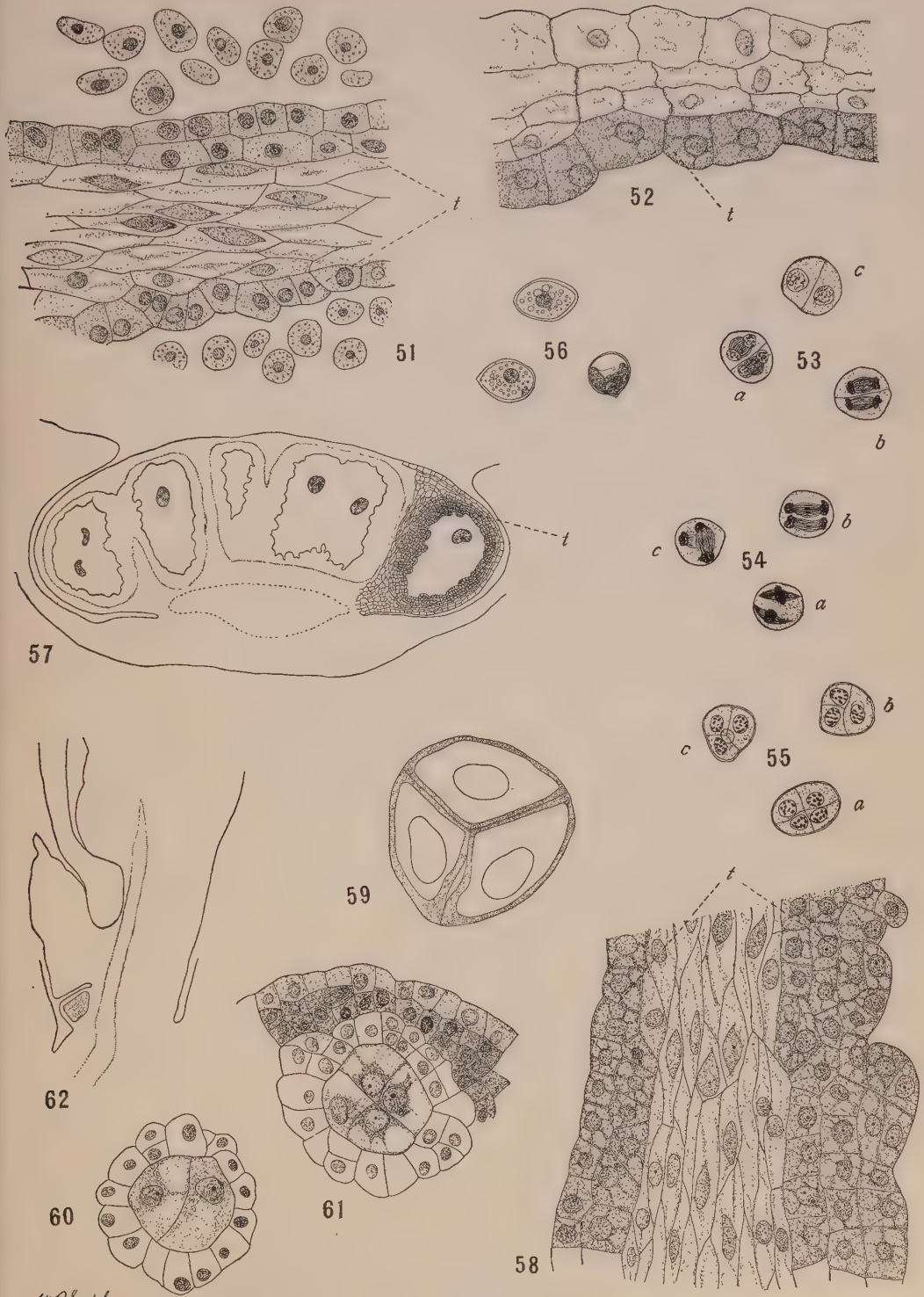

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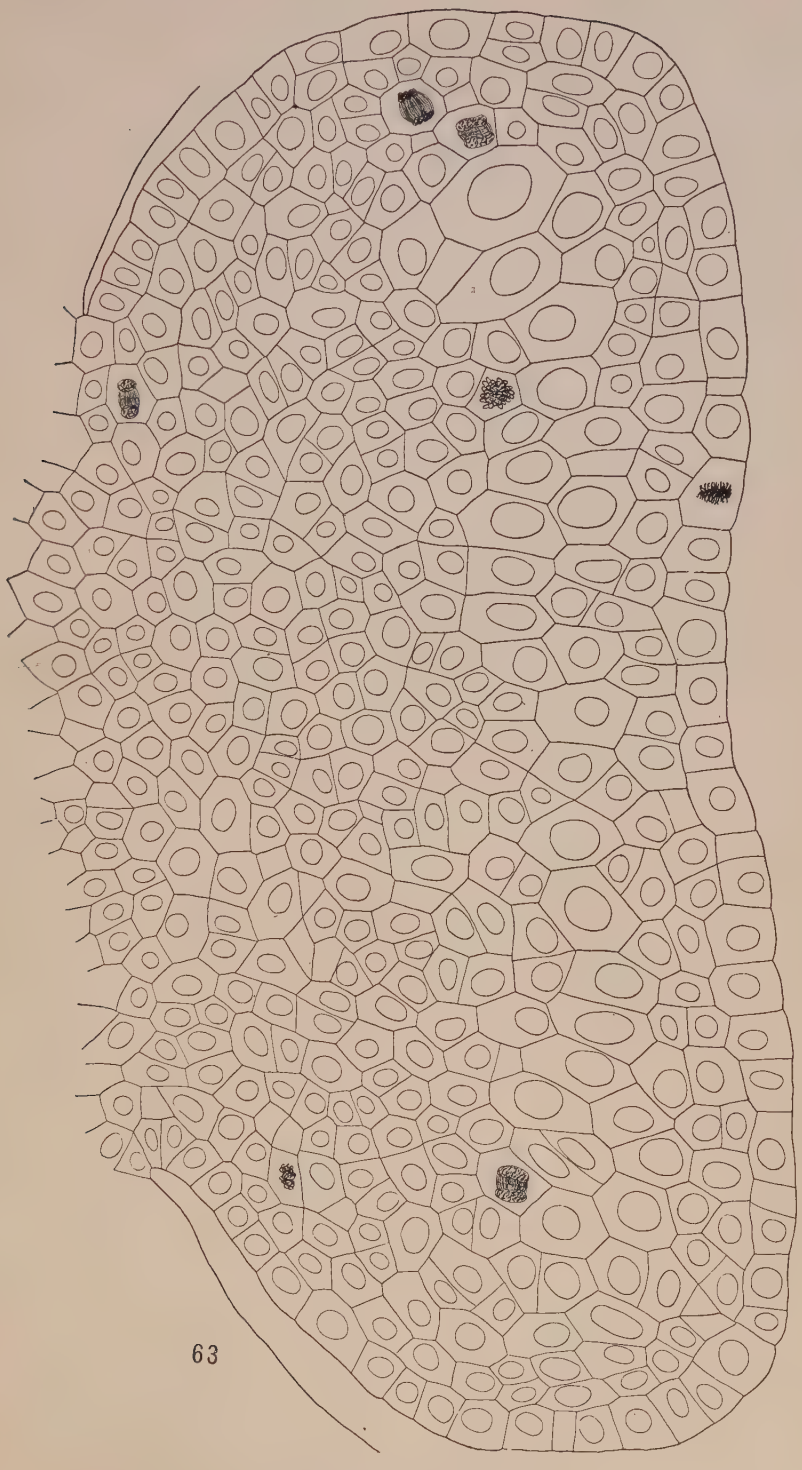

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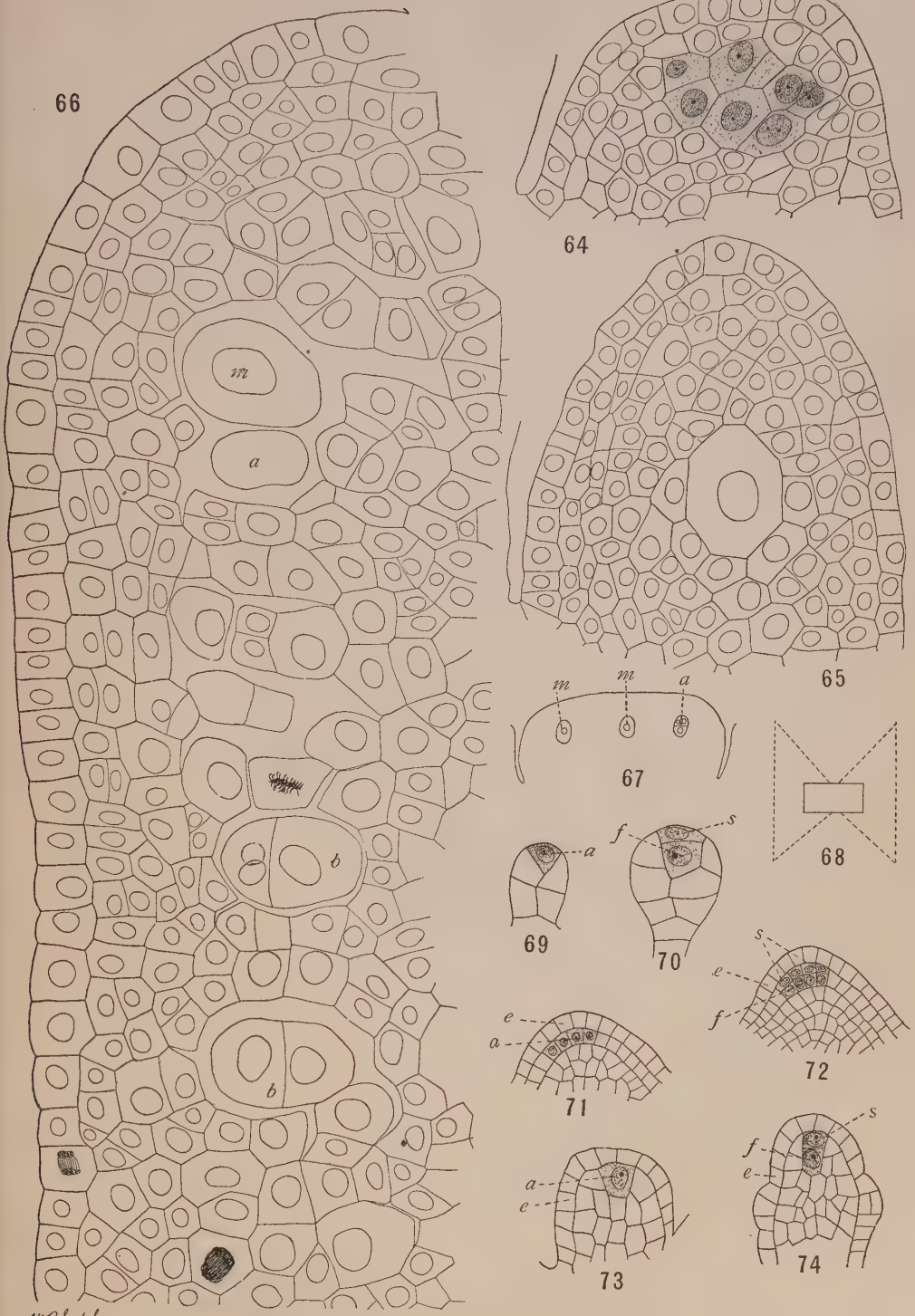

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SMITH on ISOETES

THE STRUCTURE AND DEVELOPMENT OF THE SPOROPHYLLS AND SPORANGIA OF ISOETES.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. XVIII.

R. WILSON SMITH.

(WITH PLATES XIII–XX)

(*Concluded from page 258*)

THE SUCCESSION OF SPOROPHYLLS.

THE order of succession of the sporophylls is subject to some variation. It is not at all uncommon, especially in *I. Engelmanni*, to find the regular sequence interrupted by the occurrence of several megasporophylls among the microsporophylls. Occasionally, also, sporangia are found containing both megaspores and microspores. This is rarely the case in wild plants, though quite common, along with other irregularities, in those cultivated in the laboratory. Some plants taken in December, after growing rapidly for seven or eight months in the laboratory, had formed only megasporangia; some others, though producing a few microsporophylls, had failed to bring any microspores to perfection.

The sterile leaves of *I. echinospora* differ from the fertile ones chiefly in their smaller size, the reduction of the sheathing base, and the absence of a developed sporangium. They remain green throughout the winter; while the sporophylls, set free by decay of the base and buoyed up by the gas within the numerous air cavities, are borne away by currents or waves. A close study of the sterile leaves almost always reveals the presence of aborted sporangia. These range in size from a few to many hundred cells; they are often of irregular shape and have lost their protoplasmic contents, though now and then one is found in which a few spores have matured. A longitudinal section of a typical sterile leaf is shown in *fig. 62*, in which the shaded part

represents the undeveloped sporangium. The sterilization of the sporangium does not affect the development of the velum, a fact which supports Hofmeister's view of the primary separation of velum and sporangium. The occurrence of aborted sporangia on so many of the sterile leaves shows that all the leaves are potentially sporophylls, and suggests the probability that Isoetes has retained a more primitive form of the sporophyte than any other vascular plant.

HOMOLOGY OF THE ARCHESPORIUM.

The term "archesporium" was first employed by Goebel (1), who defined it as a cell, cell-row, or cell-plate, from which all the spore-producing cells are formed, and who concluded that in all sporangia the archesporium occupies a hypodermal position. Allusion has been made to the difficulty of accepting this conclusion in such a case as that of Isoetes, but the difficulty is not peculiar to Isoetes. Bower has shown that in several pteridophytes (Selaginella, Equisetum, Lycopodium) the archesporium is not delimited by the first periclinals of the outer layer. How shall we define the archesporium in cases where there is no single hypodermal layer from which the whole mass of sporogenous tissue is derived, and to which the term can be correctly applied as required by etymology and definition? We must either modify our conception of the archesporium or abandon the term altogether as failing to express the facts. It appears to the writer that by changing our notion of the necessary position of an archesporium we could not only avoid this difficulty but would also be enabled to make a more consistent comparison of the sporangia of seed-plants and pteridophytes than is possible with the present nomenclature.

It is pretty generally recognized that there is no true epidermis in pteridophytes. The so-called epidermis is physiologically but not morphologically equivalent to that of seed-plants, for a true epidermis is traceable to a primary layer of the embryo, the dermatogen, which is distinctly present only in seed-plants. As the dermatogen is not represented in pteridophytes, unless it

be in the root-tip, there is consequently no true epidermis, and the part which performs the functions of an epidermis is the outermost layer of the periblem derivatives. Thus, while spermatophytes have three embryonic tissue regions, the plerome, the periblem, and the dermatogen, pteridophytes, with the possible exception of the root-tip, have only the two first-named regions.

The archesporium of all spermatophytes is hypodermal. In no case is it known to be derived from the superficial cells. The epidermis is from the beginning distinct from the inner cells of the sporangium. Though there may be periclinal divisions in the superficial cells, as in gymnosperms, many Ranunculaceæ, etc., these occur only after the differentiation of the archesporium, and the cells so added merely increase the thickness of the wall or apex of the sporangium, but never become part of the sporogenous complex. The true epidermis, set apart at a very early period from the inner tissue of the embryo, is incapable of producing spore-forming cells. That rôle is played by special cells of the outer layer of the periblem.

It is otherwise with the "hypodermal archesporium" of pteridophytes. This is cut off by periclinal divisions from a superficial cell in the case of ferns and Equisetum, and from a group of such cells in the case of the Lycopodiales and Isoetes.

But if the absence of a true epidermis in pteridophytes and the homology of periblem with periblem in all vascular plants are conceded, then the hypodermal cells of spermatophytes are comparable, not to the hypodermal cells of pteridophytes, but to the superficial cells. As we have seen, the sporogenous mass in pteridophytes can always be traced to superficial cells, but in spermatophytes to hypodermal cells and no further; that is, in all cases the function of giving rise to spore-producing cells is localized in certain cells of the outer layer of the periblem.

These facts serve to show the inconsistency of undertaking to limit the archesporium to a hypodermal position in all cases. The cell or group of cells whether superficial or hypodermal, to which in a last analysis all the sporogenous portion of a sporangium can be traced, ought to be called the archesporium. The

change of nomenclature which I would propose, therefore, refers only to the position of the archesporium, and not at all to the meaning of the term. It is simply this: let the term archesporium continue to be used as at present in descriptions of seed-plants, but let it be understood in the case of pteridophytes to signify the superficial cell or cells from which the spore-forming tissue is derived. In this way the difficulties which have been pointed out will all be met, and a better system of homologies can be made for sporangia in general. The archesporium will always occupy the same position relative to the primary meristematic regions, and will be the only part from which the spore-forming tissue arises.

The nomenclature here proposed can be more easily understood by reference to the accompanying diagrams. *Figs. 69* and *70* represent two early stages of the sporangium of a common fern (Pteris), but for the present purpose may be taken as typical of any pteridophyte sporangium. The shaded cell (*a*) would be regarded as the archesporium; it divides into an inner fertile cell (*f*), from which all the spore mother cells are derived, and an outer cell (*s*) which gives rise to a large part of the sporangium wall. In some instances, as we have seen, the separation into fertile and sterile cells is not accomplished by the first division. In such cases there is no contradiction of terms, since all the spores arise from the archesporium. The final condition is the same in all cases, the difference consisting simply in the earlier or later sterilization of the wall region.

Fig. 71 represents a young microsporangium and *fig. 73* a young megasporangium of an angiosperm. The outer layer (*e*), the epidermis, takes no part in the formation of the spore producing cells; *a* is the archesporium, which usually, as in pteridophytes, divides into an outer sterile region (*s*) called the primary tapetum, and an inner fertile region (*f*) called the primary sporogenous cell or cells (*figs. 72, 74*). The name primary tapetum was given to the sterile region to express its supposed function of giving origin to the true or functional tapetum. Enough is now known of the origin of the true tapetum to enable us to say

it has no definite relation to the primary tapetum, and that in fact the term "primary tapetum" is a misnomer. The true tapetum, in many cases at least, is not represented by any morphological structure in the young sporangium.

Is it not possible that the cells *s s* of *figs. 72, 74* represent the wall layer of *fig. 70*, and that the "primary tapetum," in addition to the protective and sometimes nutritive purpose which its derivatives subserve, has also a phylogenetic meaning as a survival of the pteridophyte sporangium wall which has been in great part replaced by the true epidermis? Such at least is the view suggested by a comparison of embryonic organs in general, and of the relations of the primary sporogenous cells.

RELATIONS OF THE VELUM.

On the question whether the velum has any homologue among other plant structures my observations do not furnish any information. It has been compared on the one hand with the indusium of ferns, and on the other hand with the integument of an ovule. The possibility of the latter relation certainly has not been disproved, but the evidence for it is so scant that it must remain merely an interesting suggestion. As to the other relationship, it ought to be borne in mind that the only ferns which can be at all closely related to Isoetes are the eusporangiate families, and all of these bear naked sporangia. The indusium appears in fact to be a special organ of the higher leptosporangiate ferns, without representation in the lower families, such as the Osmundaceæ or in the eusporangiates. This absence of an indusium in the intermediate orders, and the doubtfulness of the homology of the various outgrowths known as indusia, make it impossible to regard the velum and indusium as more than homoplastic structures.

THE AFFINITIES OF ISOETES.

The systematic position of Isoetes has been discussed again and again. By Linnaeus it was placed among the vascular cryptogams, where most later taxonomists have been content to leave it. During the first half of the present century it was most frequently

grouped with the Marsiliaceæ and Salviniaceæ, chiefly on the grounds of their heterospory and hydrophytic habit. DeCandolle was the first to suggest a connection with Lycopodium. In this view he was followed by Brogniart, Endlicher, Hofmeister, and the later German botanists. A summary of the various relationships which have been assigned to Isoetes was given in 1888 by Vines (1), who in the same article put forth the opinion that its affinities are with the eusporangiate ferns, rather than with the Lycopodiales. More recently Farmer (1) and Campbell (4) have expressed their concurrence with this disposition of the genus.

Since this classification has been retained by Vines in his *Text-Book of Botany*, and adopted by Campbell in his *Mosses and Ferns*, it will not be unprofitable to re-examine the evidence, with the purpose of seeing what light can be thrown upon the subject by the present and other recent investigations.

In any discussion of relationships, and especially when there is so great diversity of opinion as in the present case, the conclusion is likely to be a personal one merely, dependent on the kind of evidence which the examiner holds most weighty, rather than on its absolute nature. There are some general principles, however, to which everyone will probably assent, and which ought to govern one in estimating the relative value of the conflicting evidence on which the taxonomist relies. In the first place, the larger the number of characters in which there is agreement, the closer is the relationship, especially if the characters are such as are known to have great taxonomic value in groups related to the one under consideration. Of single characters, those which are most constant are of most value, even though we are not able to detect their special utility. It is generally accepted, too, that those characters which appear in the embryonic stages of an organism serve best to mark its wider relationships, as of class or family, while characters which do not display themselves till later in the individual life are better adapted to distinguish the near relationships of species and genus. This principle applies not merely to the organism

as a whole, but quite as fully to the embryonic stages of its different organs, such as leaf, root, sporangium, and the like.

In conformity with these principles it is proper, in the determination of natural affinities, to place great emphasis upon the reproductive parts, for such parts are found to show very great constancy in their form and occurrence. The sporangia especially, and the form and arrangement of the sporophylls, have long been recognized as of the highest importance. Thus, the classification of the Filicales is largely based on sporangial characters; and the position of the Salviniaceæ and Marsiliaceæ, which was formerly as unsettled as that of Isoetes, was established beyond doubt as soon as the development of the sporangia was fully understood.

It is chiefly on the basis of the superficial resemblances of the sporangia of Isoetes with those of Lycopodium and Selaginella that it has been so long associated with them. If we enumerate the chief differences between the sporangia of Lycopodiales and of ferns, we shall see that in every particular Isoetes agrees with the former. While the Filicales bear numerous sporangia on the dorsal surface of the leaf, Isoetes and the Lycopodiales, with the exception of the Psilotaceæ, the exact relation of whose sporangia to the leaf is still in dispute, bear but one sporangium to a sporophyll, and that on the ventral surface at the base. Such exceptional forms as the Ophioglossaceæ and Marsiliaceæ do not help us in this inquiry. Though it may be true that the whole sporangiophore of the Ophioglossaceæ is, as several morphologists have suggested, the homologue of the single sporangium of Lycopodium or Isoetes, the suggestion is so hypothetical in itself as to give no support to any view based upon it. It is only in the position of the sporangium that these families approach Isoetes; in other sporangial characters, such as number and development, they are like other ferns.

The relative age of the leaves, when the sporangial rudiments first make their appearance, is of considerable significance. In the Filicales, with the exception of the heterosporous forms,

which being leptosporangiate cannot be closely related to Isoetes, the sporangia appear late in the history of the leaf. There is an enormous development of the midrib with its conductive tissues, and of the expanded pinnæ, before the sporangia are recognizable. Nothing is more striking, however, than the quickness with which in the Lycopodiales and Isoetes the rudiment of the sporangium follows the inception of the leaf, which when the sporangium first comes into view is no more than a mere papilla of undifferentiated tissue, without a sign of photosyntactic or conductive tissue.

Still more far-reaching is the agreement of Isoetes with the Lycopodiales in the character of the sporangium rudiment. Goebel (1) in his celebrated paper of 1880–1 classified sporangia as leptosporangiate or eusporangiate according as they arise from single cells or from groups of cells. Though the two classes are connected by transitional forms, such for instance as the Osmundaceæ, in which the sporangia, though always classified as leptosporangiate, do not arise strictly from single cells, the distinction has been approved by all later morphologists. The leptosporangiate plants make a well-defined and consistent group, but the eusporangiates comprise very diverse forms, including the several divisions of seed-plants, the Lycopodiales, the Equisetales, and part of the Filicales. If, however, we leave out accessories, and turn our attention entirely to the essential part of the sporangium, that is to the sporogenous tissue, we find a distinction which has the merit of leaving the Filicales an unbroken group, and of agreeing closely with what is required by a consideration of other characters. This distinction pertains to the origin of the archesporium. The spore-forming part of the sporangium of Isoetes and Lycopodiales can be traced back to a number of cells placed transversely to the leaf, but of all other pteridophytes to a single cell. Is not this distinction as valid as that which pertains to the origin of the whole sporangium? If so, it tends strongly to justify the inclusion of the Lycopodiales and Isoetes within a distinct group set apart from all other vascular cryptogams.

Certain other features of the sporangium of Isoetes find duplication only among members of the Lycopodiales. In all the higher leptosporangiate ferns there is an elaborate mechanism for the bursting of the sporangium and the scattering of the spores. This device, consisting of a row of peculiarly thickened cells (the annulus), and a group of cells which form an easy place of rupture (the stomium), is very rudimentary in the lower leptosporangiates (Osmundaceæ), and in the Ophioglossaceæ and Marattiaceæ, but it is not altogether absent. There is at least a predetermined line along which dehiscence shall take place. The elaboration of this dehiscence apparatus is one of the chief peculiarities of the higher leptosporangiates. When we turn to the Lycopodiales and Isoetes, however, we find positively no contrivance for dehiscence, and no vestige of an annulus or stomium. The sporangium wall is simple, and bursts by desiccation in Lycopodium and Selaginella, and by decay in Isoetes; and neither method can be regarded as a specialization.

Another analogy has been brought to light by Bower's discovery in Lepidostrobus of certain radiating strands or processes in the sporangium which are regarded by him as very probably of the nature of trabeculæ. Since the relationship of Lepidostrobus to Lycopodium can hardly be doubted, there is here a point of contact with this group of plants in a feature in which otherwise Isoetes stands alone.

Again, Selaginella and Isoetes agree very nearly in the manner of selection of the megaspore mother cells. The unselected mother cells do not divide at all, and all the spores resulting from the division of the fertile ones as a rule reach maturity. In heterosporous ferns all the mother cells divide into spores, of which but one becomes a megaspore. The contrast may be expressed in the statement that the megasporangium is differentiated in Isoetes and Selaginella *before* the tetrad division, but in heterosporous ferns not until *after* that division.

The persistence of the tapetum in Lycopodiales and Isoetes is a character to which no great importance is to be attached, for

tapetal characters are notoriously variable. Such bearing as it has, however, is in harmony with what may be inferred from other features of the sporangium. It involves no disorganization of the cells, no multiplication of nuclei except as related to cell-division, and no mingling of naked protoplasm with the young spores.

One of the facts which Vines advanced as an argument against the usually accepted classification of Isoetes is the absence of a strobilus, the characteristic arrangement of the sporophylls in the Lycopodiales. He contrasts also the elongated, slender, branched stem of Lycopodium or Selaginella with the short unbranched stem of Isoetes, which much more closely resembles that of some eusporangiate ferns. It may be doubted whether such superficial characters, unless accompanied by internal features of which they are the outward expression, have any value in settling the relationship of distinct genera or families. At all events, their usefulness in angiosperm taxonomy is limited to the distinction of species; they would be of no use in deciding the family to which an undetermined species ought to belong. I am inclined to think the whole plant-body of Isoetes can best be explained as a shortened strobilus, just such as Lycopodium would become by suppression of the stem and axis, while allowing a normal development of the leaves and sporangia.

The most obvious diagnostic character of the three groups of pteridophytes is furnished by the leaves. The leaves of the Isoetes are *sui generis*, and afford little ground for associating it with any one group rather than another. Though they are relatively few and large, as is the case among ferns, their unbranched outlines and simple tissues show an analogy with the leaves of Lycopodiales; while their peculiar vascular bundles, and chambers, and diaphragms remove them as effectually from either group. There is record, it is true, of a fossil Isoetes with a branched leaf, indicating, when taken in conjunction with the sudden reduction of the vascular bundle just above the ligule, the possibility that the present form of the leaf may be a reduced one representing a more complex ancestral type. But

we must admit, so far as mature leaf structures are concerned, that Isoetes occupies an isolated, and in no sense an intermediate position.

The testimony of the young leaves, however, is not so neutral. The form of the leaf rudiments, their manner of growth, and arrangement about the axes are the same in Isoetes as in Lycopodium and Selaginella, and quite different from what is seen among ferns. The difference is not fully expressed in saying that in one case the leaf originates from a single apical cell, and grows by means of it, and that in the other case the initiative is from a group of cells. The leaves of ferns are distinctly acrogenous, which method of growth gives them the power of assuming complex forms and allows the successive and often slow formation of stipe, pinnæ, and pinnules, and their gradual unfolding. A leaf which grows as does that of Isoetes has its power to assume a complex form limited to the time when it is meristematic throughout; as soon as the apex becomes permanent tissue the outline of the leaf is determined. The difference between such leaves is fundamental and far-reaching. A Lycopodium leaf could easily attain the size of an Isoetes leaf by retaining the meristematic power for a longer time, for they differ only in degree. The leaf of a fern could become like that of Isoetes, or *vice versa*, only by a radical change in the manner of growth.

The similarity of the leaf rudiments of Lycopodium and Isoetes is only a particular instance of a general likeness which extends to all their embryonic organs. We have already seen how this is true of the sporangia; and it holds equally good for the roots[1] and stem apex. In none of these organs is there ever an apical cell or any concentric segmentation of the apices, such as are characteristic of all the Filicales and Equisetum. A difference in this respect in the case of apical-growing organs, like the roots and stem, may not lead to important differences in the mature structures, as the variation in the stem apices of Selaginella suffices to show. But a comparative examination of

[1]Van Tieghem (1), but Bruchmann (1) entertains a different view.

meristems was shown by Bower to possess considerable phylogenetic value, in the case of ferns, and to lead to results which agree with those arrived at by a comparison of other characters. The fact that Bower has since changed his view with regard to which type of fern is more primitive does not in any way lessen the value of his previous conclusions. If we extend the series made out by him it would be in this order: typical leptosporangiate ferns, Osmundaceæ, eusporangiate ferns, Selaginella, Isoetes, and Lycopodium. In this connection the dichotomy of the roots of Isoetes, Lycopodium, and Selaginella ought not to be overlooked.

The ligules of Selaginella and Isoetes were by Goebel made the ground for grouping the two genera into one order, the Ligulatæ, though the classification was recognized by its proposer as merely one of convenience. In the former part of this paper I have made a comparison of these organs, and expressed the view that their similarity is sufficient to demonstrate their homology. If this view is correct, it furnishes additional support to the relationship of Isoetes and the Lycopodiales, especially in consideration of the discovery of a ligule in the vegetative leaves and the sporophylls[2] of Lepidostrobus, another lycopodiaceous plant.

Turning now to the gametophytes, we notice that when Vines suggested the connection of Isoetes and ferns, it was supposed that important differences existed between the female gametophytes of Isoetes and Selaginella; but the later and more complete investigations of Heinsen (1) and Arnoldi (1) have demonstrated their close resemblance. The diaphragm of the female gametophyte of Selaginella is not a true septum, and does not arise as Pfeffer (1) supposed it did, by the division of the spore into two cells. In both Isoetes and Selaginella, the free division of nuclei, their parietal placing, and the gradual extension of cell division from the periphery to the center of the spore are the same, and have no counterpart in the germination of the megaspores of heterosporous ferns. The gametophytes agree also in the absence of chlorophyll.

[2] Maslen (1).

Nearly similar evidence is furnished by the male gametophyte. Though Belajeff (1), to whom we owe the most exact investigation of the subject, says the male gametophytes of Isoetes and Selaginella afford little ground for relating the two genera, he has shown several points of resemblance, such as the separation of the prothallial (or rhizoidal) cell from the single antheridium by a cellulose wall, and the final dissolution of the non-cellulose septa of the antheridium wall, so that the spermatozoids float free in the cavity of the spore.

Though not disposed to place much dependence as a clue to the working out of phylogenetic relationships among heterosporous plants on such structures as archegonia and antheridia, which must necessarily conform more or less in shape to the space in which they are confined, I find some interest in the fact that Isoetes and Lycopodium are the only genera of pteridophytes in which the occurrence of more than two neck canal nuclei has been reported, and that in Isoetes, as in *Lycopodium Phlegmaria* and Equisetum, the plane of the division of the primary neck canal nucleus is at right angles to the archegonium axis.

The two characters which stand most in opposition to the inclusion of Isoetes in the group Lycopodiales are its multiciliate spermatozoid and the embryogeny of its sporophyte. Campbell has very properly emphasized the similarity of the Isoetes spermatozoids to those of ferns. It requires only a brief survey of the plant kingdom to show the great constancy of the form and behavior of male cells in different classes of plants. Consider, for example, the non-motile spermatozoids of the Florideæ, or the biciliate spermatozoids of bryophytes. Accordingly, if we still classify Isoetes among Lycopodiales, we must admit that the multiciliate spermatozoids make an exception to a constancy which is otherwise remarkable. Unfortunately, we have only Lycopodium and Selaginella for comparison, and are still in ignorance as to what the gametophytes of the other genera may have to tell us.

The embryo of Isoetes finds its nearest approximation in Botrychium[3] though the resemblance may be only an external

[3] Jeffrey (1).

one, due to the late differentiation of the embryonic organs and the suppression of the stem, rather than to any deep-seated likeness. However that may be, the suspensor of Lycopodium and Selaginella is a positive morphological character separating them from Isoetes. Probably the embryos of Isoetes and Botrychium can be looked upon as generalized types, the specialization taking the form in ferns of a very early demarcation of the embryonic organs, and in Lycopodium and Selaginella of a suspensor.

Two other possible reasons for relating Isoetes to ferns deserve a passing mention. Of the connection between the velum and the indusium enough has already been said; and of the agreement of the stems of Isoetes and Botrychium it is sufficent to say that the agreement is simply in the fact of secondary thickening.

The claim that Isoetes is the genus of modern pteridophytes which makes the closest approach to angiosperms, particularly to monocotyledons, gives it an interest quite out of proportion to its numerical representation. It is not clear, however, that the claim is well supported by facts. Unquestionably Isoetes and Selaginella, in their heterospory, and their intrasporic and reduced gametophytes, exhibit features of life history which run closely parallel to that of seed-plants; but such features really foreshadow monocotyledons no more than they do other seed-plants. The hypodermal archesporium, and the origin of the megaspore mother cell as the lowest of a row resulting from periclinal divisions of an archesporial cell—two points which my observations disprove — would, if established, be as strong proof of a gymnosperm as of an angiosperm attachment. Some facts distinctly favor the gymnosperm connection; these are the manner of germination of the megaspore, and the method of selection of the megaspore out of a large mass of potentially spore-producing cells, as in Cycas, Callitris, etc., to which may be added whatever favors the relationship of Isoetes with the Lycopodiales.

Professor Campbell has shown that the embryo of Isoetes bears a likeness to that of a monocotyledon in having a lateral

stem apex and a terminal cotyledon, and suggests a comparison with the embryo of Alisma for instance. The resemblance in form is undoubtedly very close, but we ought not to overlook some equally important differences. The entire absence of a suspensor in Isoetes, which has been brought forward as an objection to its close affinity with Lycopodium, militates quite as strongly against an affinity with monocotyledons; and the foot, which is particularly well developed in Isoetes, cannot be said to have any clear representative in monocotyledon embryos.

In general habit Isoetes has been compared to some grasses, rushes, and the like; this is a mere external resemblance in one of the most adaptive features of plants, and not supported by internal and essential similarities. A similar objection can be raised to the comparison of the stelar regions of Isoetes and of such monocotyledons as Dracæna. In the latter it is true there is a secondary thickening carried on by means of an extra stelar "cambium," but this cambium merely adds parenchymatous tissue within which separate vascular bundles are organized; there is nothing strictly comparable to the prismatic zone or central xylem cylinder of Isoetes. Even were the likeness much closer than it is, the peculiar stem of Dracæna, Yucca, etc., is so certainly a newly acquired, and not a primitive character, that it affords no sound reason for deriving monocotyledons through an Isoetes-like type.

To one who has followed this discussion thus far it will be evident that in the writer's opinion the balance of evidence is in favor of relating Isoetes to Lycopodium and Selaginella rather than to eusporangiate ferns. Of course the facts are not all in hand as yet, and new discoveries may materially affect the aspect of the case. The facts which the present investigations have brought to light certainly tend in the one direction. The mode of origin, development, position, and general characters of the sporangia, the development of the leaf, and the nature of the ligule point to the correctness of including Isoetes among the Lycopodiales; while the form of the spermatozoids and

embryo show the necessity of making it a separate family. The Lycopodiales so constituted comprise six genera pretty widely separated in morphological characters, as from the antiquity of the group one might naturally expect. But the extreme differences are not greater than in the Filicales. If we can include Azolla, Marsilia, the common ferns, Hymenophyllum, the Marattiaceæ, and the Ophioglossaceæ in one group, it ought not to appear inconsistent to include Psilotum, Lycopodium, Phylloglossum, Selaginella, and Isoetes in a group of coordinate rank. A fuller knowledge of the three little-known genera may tend to confirm this view, especially if they depart as widely from the remaining genera in other characters as in general habit and sporangia; but if their gametophytes, spermatozoids, and embryos agree very nearly with Lycopodium and Selaginella, it will probably be better to make of Isoetes a fourth group of pteridophytes equivalent in rank to the three now universally recognized. If an affinity with seed plants must be sought, the evidence points to a connection with gymnosperms rather than with monocotyledons.

SUMMARY.

1. The stem apex lies at the bottom of a funnel-shaped depression, around the sides of which the leaves are arranged spirally. This depression is produced by the expansion of the cortical cells of the stem in all directions.

2. The leaves arise as crescent-shaped bands of meristematic tissue. At first the basal part of the leaf (the sheath) grows most rapidly; afterwards the region of growth is transferred to the part above the ligule. There is no persistent or sharply-marked zone of meristem. The whole leaf is meristematic at first; it then gradually passes into permanent tissue, the change beginning at the apex and extending gradually downwards.

3. The air-cavities are formed out of four longitudinal bands of cells, which after losing their contents and power of multiplication are ruptured into transverse partitions by the growth of the other parts of the leaf. The size, but not the number of the air-cavities, increases with the age and growth of the leaf.

4. The ligule originates in a single vesicular cell as described by Hofmeister. The mature ligule can be distinguished into four regions: (1) the sheath which has its origin in the lowermost cells of the young ligule, (2) the glossopodium, (3) a region of living cells, and (4) a region of disintegrating cells.

5. The rudiment of the sporangium is a transverse row of superficial cells below the ligule; the upper part of this gives rise to the velum, the lower part to the sporangium proper.

6. There is no definite hypodermal archesporium. The middle cells of the sporangium *Anlage* are the first to undergo periclinal divisions. Additions to the sporogenous complex are made from the superficial cells of the sporangium.

7. The general direction of growth of the sporangium is at right angles to the face of the leaf, with a slight tendency in young sporangia to an upward direction. The cells are not in well-arranged rows or stratified layers.

8. There is no evidence that certain of the archesporial cells give rise to trabeculæ only, and certain others to mother cells only. The trabeculæ and megaspore mother cells or groups of microspore mother cells greatly outnumber the archesporial cells.

9. There is no evidence that each of the primary cells of the sporangium pursues an independent growth. On the contrary, their derivatives blend indistinguishably.

10. The microsprangia and megasporangia are indistinguishable until they have attained a volume of 15,000–25,000 cells.

11. The sporangium becomes recognizable as a microsporangium by its differentiation into irregular deeply-staining and feebly-staining radial bands. The deeply-staining regions after a period of active multiplication become the mother cells. The feebly-staining regions become the trabeculæ, walls, and tapetum,

12. The tapetum is organized out of the layer of sterile cells adjacent to the mother cells; its cells are small, densely cytoplasmic, and persistent.

13. The middle cells of the trabeculæ become elongated by compression and growth; their nuclei also become elongated and spindle-shaped.

14. The outer wall of the microsporangium is usually four layers thick, the innermost layer being part of the tapetum. The inner wall, that is the cells between the base of the sporangium and the vascular bundle, is probably formed by sterilization of cells derived from the primary cells of the sporangium.

15. The divisions of the microspore mother cells may be either successive or simultaneous. The two spindles of the second division do not become connected by secondary fibers. The microspores are usually bilateral but sometimes tetrahedral.

16. The number of microspores in a sporangium is 150,000–300,000.

17. A sporangium first becomes recognizable as a megasporangium by the marked enlargement of many or most of the cells of about the third and fourth layers. All such enlarged cells are to be regarded as potential mother cells, and the number of them which succeed in producing megaspores is probably dependent on nutrition. No tabular tapetal cells are cut off in connection with the development of the megaspore mother cells, nor is the megaspore mother cell the innermost of a row of cells formed from a single archesporial cell in a manner comparable to what is seen in the ovules of seed-plants.

18. Many cells which enlarge almost to the size of mature mother cells are finally unable to give rise to spores, but divide up into smaller cells which ultimately form part of the tapetum.

19. The trabeculæ, tapetum, and walls arise in the megasporangium as in the microsporangium, the chief difference being the greater massiveness of the single trabeculæ in the former and the much greater abundance of the tapetum.

20. No details of the division of the megaspore mother cell were obtainable. The megaspores are usually tetrahedral in arrangement, but occasionally bilateral. The number in a sporangium is 150–300.

21. The first leaves of a season are megasporophylls, and these are succeded by microsporophylls. There is occasionally some irregularity in the order of succession, and sometimes a sporangium is found which bears both kinds of spores.

22. The sterile leaves in a majority of cases have aborted sporangia. When these have made any considerable development they are usually found to show the characters of megasporangia.

23. The sporangia after all cell divisions have ceased continue to increase in volume, apparently by the osmotic properties of the substances surrounding the young spores.

24. An attempt to relate the change from megasporophylls to microsporophylls to an exhaustion of the nutritive cortical cells formed in the preceding year was unsuccessful.

25. To secure a more consistent nomenclature it is proposed to employ the term archesporium in speaking of a pteridophyte sporangium to designate the superficial cell or cells from which the sporogenous tissue takes its origin.

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EXPLANATION OF PLATES XIII–XX.

The drawings, except *fig. 1*, have been made with the aid of a camera lucida, and all have been reduced by photography to two fifths of their original size. Both in the text and in the explanation of the plates, the terms transverse, longitudinal, and tangential, when used to describe sections of the ligule and the sporangium are to be understood as indicating the planes in which the sporophylls were sectioned.

FIG. 1. Base of microsporophyll, inner face showing the sporangium (*s*), the velum (*v*), and the ligule (*l*). × 4.

FIG. 2. Radial longitudinal section of base of sporophyll ; *s*, *v*, *l*, as in *fig.* 1. × 5.

FIG. 3. Longitudinal section of apex of a small plant. × 48.

FIG. 4. Longitudinal section of apex of a larger plant ; *x*, the tracheids, *p*, the prismatic layer, *t*, the leaf traces, *m*, the cambium (semidiagrammatic). × 48.

FIG. 5. Part of the prismatic ring as seen in transverse section of the stem ; *x*, *p*, *t*, *m*, as in *fig. 4*. × 160.

FIG. 6. Cortical cells from the neighborhood of the cambium. × 240.

FIG. 7. Cortical cells from the outer region of stem. × 240.

FIG. 8. Longitudinal section of a number of young sporophylls ; the sporangia are indicated by the dotted outlines. × 48.

FIG. 9. Cross section of a young leaf above the ligule. × 300.

FIG. 10. Cross section of a young leaf more advanced than that shown in *fig. 9* ; the position of the future air chambers is shown by the groups of nearly empty cells ; the small circles of *figs. 9* and *10* indicate the side towards the axis of the plant. × 300.

FIG. 11. Part of a longitudinal section of a leaf more advanced than that of *fig. 10*, showing origin of the air cavities ; *p*, the phloem, *x*, the xylem. × 300.

FIGS. 12–13. Transverse section of leaf with the vesicular cell from which the ligule originates. × 300.

FIG. 14. First division of the ligule, sectioned in plane *a*—*a* of *fig. 15*. × 490.

FIG. 15. First division of the ligule seen in radial longitudinal section. × 490.

FIGS. 16, 18. First division of terminal cell of ligule sectioned in plane *a*—*a* of *fig. 15*. × 490.

FIG. 17. The same sectioned in plane *b*—*b* of *fig. 15*. × 490.

FIG. 19. Tangential section of older ligule. × 490.

FIG. 20. Transverse section of ligule of same age as that of *fig. 19*. × 490.

FIG. 21. Transverse section of still older ligule. × 300.

FIG. 22. Median radial longitudinal section of base of half-grown ligule; *s*, the sheath, *g*, the glossopodium, *v*, the velum. × 490.

FIG. 23. Radial longitudinal section of base of mature ligule at the position indicated by *a*—*b* of *fig. 25*, showing the thickened cells of the velum and leaf adjacent to the ligule. × 48.

FIG. 24. The same in *I. Engelmanni*. × 48.

FIG. 25. Transverse section of ligule and leaf at the position indicated by *a*—*b* of *fig. 23*. × 30.

FIG. 26. Median radial longitudinal section of young sporophyll, showing rudiment of the sporangium. × 490.

FIGS. 27–28. The same more advanced. × 490.

FIG. 29. Transverse section of young sporophyll with sporangium. × 490.

FIG. 30. Transverse section of sporangium more advanced. × 490.

FIG. 31. Tangential section of young sporangium. × 490.

FIGS. 32–38. Radial longitudinal sections of young sporophyll; *fig. 37* is a section through the side of the sporangium of which *fig. 36* is a median section; *v*, the velum, *g*, the glossopodium, *s*, the sheath. × 490.

FIG. 39. Transverse section of sporangium more advanced. × 490.

FIG. 40. Transverse section of leaf with sporangium of same age as that of *fig. 39*. × 490.

FIG. 41. Median radial longitudinal section of a sporangium of about the same age as the last. × 490.

FIG. 42. Longitudinal section of side of sporangium. × 490.

FIG. 43. Transverse section of a sporangium still older but in which the trabeculæ are not yet recognizable. × 490.

FIG. 44. Transverse section of young leaves and of microsporangium at the time of the first differentiation of fertile and sterile regions; the shaded portion represents the fertile region; *v*, the velum, *l*, the ligule, *f*, the vascular bundle. × 48.

FIG. 45. Oblique nearly tangential section of microsporangium. × 48.

FIG. 46. Tangential section of microsporangium. × 30.

FIG. 47. Cross section of sporophyll and microsporangium, showing the trabeculæ, tapetum, *t*, and a few microspores; *v*, the velum (semidiagrammatic). × 30.

FIG. 48. Portion of microsporangium at the time of the first differentiation of fertile and sterile regions. × 490.

FIG. 49. The same, showing portion of outer wall. × 490.

FIG. 50. Portion of an older microsporangium, showing differentiation of sterile regions into trabeculæ and tapetum (*t*). × 490.

FIG. 51. Portion of trabecula, tapetum (*t*), and young spores of a microsporangium. × 490.

FIG. 52. Part of outer wall and tapetum (*t*) of nearly mature microsporangium. × 490.

FIGS. 53–55. Division of mother cells to form microspores; *fig. 53* illustrates successive division; *fig. 54*, simultaneous division; *fig. 55*, the shape of the spores, bilateral in *a* and *b*, tetrahedral in *c*. × 490.

FIG. 56. Young microspores. × 490.

FIG. 57. Cross section of megasporangium with young spores and tapetum (*t*). × 48.

FIG. 58. Portion of a trabecula and tapetum (*t*) of a megasporangium, × 490.

FIG. 59. Tetrahedral arrangement of young megaspores. × 490.

FIGS. 60–61. Successive division of megaspore mother cells, spores bilateral. × 490.

FIG. 62. Median radial longitudinal section of sterile leaf with aborted sporangium (shaded).

FIG. 63. Transverse section of megasporangium first distinguishable as such. × 490.

FIG. 64. Part of transverse section of a megasporangium with a group of potential mother cells. × 490.

FIG. 65. The same with a single mother cell. × 490.

FIG. 66. Part of a transverse section of a megasporangium; for explanation see text. × 490.

FIG. 67. Diagram of young megasporangium.

FIG. 68. Diagram of a longitudinal section of the stem. See p. 228.

FIGS. 69, 70. Early stages of the sporangium of a fern. Diagrammatic. *a*, archesporium; *f*, fertile sporogenous cell; *s*, sterile wall cell.

FIGS. 71, 72. Early stages of a microsporangium of an angiosperm. Diagrammatic. *a*, archesporium; *e*, epidermis; *s*, primary tapetum; *f*, primary sporogenous cell or cells.

FIGS. 73, 74. Early stages of a megasporangium of an angiosperm. Diagrammatic. Letters as in *figs. 71, 72*.